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Morphological Studies in the Genus Nocardia

I. Developmental Studies[1]

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INTRODUCTION

The morphological criterion for separating the genus *Nocardia* (*Proactinomyces*) from true bacteria is the presence of a "mycelium" in the former. This distinction has been uncertain since little is known concerning the degree of mycelial development in *Nocardia*, and the term lacks precise definition. *Nocardia* is quite clearly separated from *Streptomyces* on the basis of the presence of aerial spores in the latter. The inadequacy of current criteria for separating *Proactinomyces* from bacteria was emphasized by Umbreit (1939). He also called attention to the fact that there are two morphologically distinct groups in the genus, which he designated as α *Proactinomyces*, the "unstable mycelial" group, and β *Proactinomyces* the "stable mycelial" group.

Interest in the actinomycetes has been stimulated by a better conception of their distribution and role in nature, and more recently, by the discovery of the production of antibiotics by certain strains (streptomycin by *Streptomyces griseus*, for example). The increasing economic importance of the group emphasizes the need for basic biological studies of their behavior. In order to determine how and to what extent mycelium is produced by various strains of *Nocardia*, it was proposed to study their development by following the growth of single cells into young colonies. It was hoped that by recording precisely the manner of growth by means of drawings, information would be gathered which would aid in clarifying the morphology and thus contribute to a better conception of the phylogenesis of the genus.

PRELIMINARY WORK

In order to obtain organisms for the morphological studies, analyses were made of 23 soils from the vicinity of Ann Arbor, Michigan, for saprophytic actinomycetes and bacteria. The method used was that

[1]From a dissertation submitted as a partial requirement for the degree of Doctor of Philosophy to the Graduate School, University of Michigan, Ann Arbor, Michigan. The author appreciatively acknowledges the guidance and encouragement of Dr. K. L. Jones, in carrying out this work.

of Jones (1943) with the exception that six replicates of each soil dilution were made instead of ten. The soils were chosen at random and in a few cases, as noted, the samples were taken at depths below the surface.

The "number" of actinomycetes in millions per gram of dry soil, the percentages of actinomycetes compared with the total of actino-

TABLE 1.—*Numbers of actinomycetes in various soils at depths indicated.*

No.	Soil Type	Depth	pH	%H_2O	*Actinomycetes*		Month collected, 1946	Strains of *Nocardia* isolated
					Millions per gram	% of Total		
	Grassland Soils							
1	Loam.....	Surface	7.0	5	1.5	14	April	3
2	"	"	7.0	5	3.5	23	"	2
3	"	2′	7.0	6	0.05	63	"	5
4	"	Surface	7.0	2	1.5	40	May	5
5	"	2′	7.0	6	0.3	3	"	6
6	"	4′	7.0	5	0.8	50	"	2
7	"	8′	7.0	1	0.2	14	"	0
	Barren Soils							
8	Sand.....	Surface	7.5	10	0.3	30	April	2
9	"	2′	7.0	8	0.3	13	"	2
10	"	8′	7.0	1	0.0003	5	"	0
11	"	Surface	7.0	5	3.6	22	July	2
	Garden Soils							
12	Loam.....	Surface	7.0	14	2.2	22	July	5
13	Clay Loam...	"	7.0	1	3.9	35	"	0
14	" "	2′	7.0	3	0.3	15	"	4
	Forest Soils							
15	Loam.....	Surface	7.0	15	4.6	27	May	0
16	"	"	7.0	28	1.4	30	"	9
17	Duff.....	"	6.0	18	0.8	19	"	2
18	Clay Loam...	"	7.0	22	0.6	11	July	2
19	Duff.....	"	5.0	5	0.2	21	"	1
	Misc. Wet Soils							
20	Mud from Swamp.....		6.0	40	1.8	39	May	2
21	" " "		6.0	37	3.8	17	"	1
22	Organic Muck.....		6.5	79	1.7	20	July	4
23	Inorganic soil under water.....		7.0	30	0.1	12	"	2

mycetes and bacteria, the pH of the soil sample, the water content, and the number of strains of *Nocardia* isolated from each sample, are shown in Table 1. Since no attempt was made to isolate all of the asporogenous, filamentous organisms there is no information present as to the relative numbers of *Nocardia* and sporulating actinomycetes (*Streptomyces*).

The "number" of actinomycetes in a gram of dry soil, in these analyses, ranged from 300 to 4,600,000 and the percentage of actinomycetes compared with the total number of actinomycetes and bacteria from 3% to 63%. *Nocardia* strains were isolated from all but four of the samples.

Sixty-one cultures of *Nocardia* were isolated from the 23 soils that were analyzed. The criteria used in determining whether the saprophytic, aerobic isolate belonged to the genus *Nocardia* were: the formation of a mycelium and the absence of conidia. The first was determined by microscopic examination of the edge of the colony, the second by culturing the isolates on enrichment medium. Bennett's[2] medium was found to be very satisfactory for this. If the isolate did not sporulate after an incubation period of two weeks it was transferred to a slant of glycerol nutrient agar and stored as a stock culture of *Nocardia* sp. The fourteen following strains were obtained from type culture collections, six of which were used in the morphological studies:

Source	Name
Centralbureau voor Schimmel-cultures, Baarn, Holland	*Proactinomyces asteroides* (Eppinger) Baldacci var. *decolor* Bald.
" "	*Proactinomyces asteroides* (Eppinger) Bald. var. *crateriformis* Bald.[3]
" "	*Proactinomyces restrictus*
" "	*Proactinomyces agnosus*[3]
" "	*Proactinomyces pseudomadurae* Bald.
" "	*Proactinomyces polychromogenes* (Vallée) Jensen[3]
" "	*Proactinomyces ruber* (Casabó) Bald.[3]
American Type Culture Collection	*Proactinomyces gardernii* 9604
" " " "	*Proactinomyces minimus* 8674
Waksman	*Nocardia polychromogenes* 3409A[3]
"	*Nocardia erythropolis* 3407[3]
"	*Nocardia maculatus* 3376
"	*Nocardia paraffinae* 3410
National Type Culture Collection, Lister Institute, London	*Proactinomyces paraffinae* 3488

Two additional cultures were furnished by Dr. K. L. Jones, and four strains were isolated by students in class work.

To avoid confusion the above designations will be used throughout this paper for the strains concerned. All would now belong to the genus *Nocardia*. However, the taxonomic questions involved in re-designation are beyond the scope of the present work. The media

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[2]Formula published with the permission of Dr. R. E. Bennett.

[3]These organisms were among those used in the morphological studies.

used in this work were: glycerol nutrient agar, Bennett's medium, Jensen's medium and Czapek's medium. The formula for each follows:

GLYCEROL NUTRIENT AGAR

Beef extract	3.0 g.
Peptone	5.0 g.
Agar	15.0 g.
Glycerol	20.0 g.
Dist. water	1000.0 cc.

pH 7.0

BENNETT'S AGAR

Yeast extract	1.0 g.
Beef extract	1.0 g.
NZ amine A	2.0 g.
Dextrose	10.0 g.
Agar	20.0 g.
Dist. water	1000.0 cc.

Adjust to pH 7.3 with NaOH

JENSEN'S MEDIUM

Dextrose	2.0 g.
Casein	0.2 g.
(dissolved in 10 cc. 0.1N NaOH)	
K_2HPO_4	0.5 g.
$MgSO_4$	0.2 g.
$FeCl_3$	trace
Agar	15.0 g.
Dist. water	1000.0 cc.

pH 6.5–6.6

CZAPEK'S MEDIUM

Sucrose	30.0 g.
$NaNO_3$	2.0 g.
K_2HPO_4	1.0 g.
KCl	0.5 g.
$MgSO_4$	0.5 g.
$FeSO_4$	0.01 g.
Agar	15.0 g.
Dist. water	1000.0 cc.

pH 6.7

METHODS

It was necessary to devise a method for studying the development of a colony of *Nocardia* from a single cell or hyphal fragment. At first a slide devised by Brown (1942) for the culture of fungi was used. A small drop of agar medium, either Czapek's or glycerol nutrient, was placed in the well in the center of the slide. After the medium hardened, the mount was ringed with vaseline, the drop of agar inoculated with a suspension of the organism, and a large cover glass superimposed. The organisms grow in a film of nutrient liquid which exudes from the agar at the juncture of the cover glass and the medium.

A single cell could be selected for study, if the inoculation was not too heavy, by focusing on the edge of the medium. The cultures were usually prepared at 10:00 P. M., incubated in moist chambers at 28° C. overnight (8–10 hours), and observations started the next morning. The cell selected for study could be located for subsequent observation by recording the settings of a mechanical stage.

After a cell had started to grow, hourly observations and drawings were made. It was necessary to select a cell that had begun to grow, because isolated cells grew poorly. Drawings were made of the developing organisms by means of the *camera lucida*, using the 4 mm. objective, magnification 800 diameters.

Because several strains failed to grow by using this method, tests were made to determine whether the difficulty was of a physical or chemical nature. Hanging drop and hanging block cultures were made using various media, but growth was erratic and unpredictable. Hanging drop mounts were completely unsatisfactory for developmental studies because it was impossible to keep the organism from shifting position.

Glycerol nutrient agar was found to give good results as a medium but apparently more oxygen was necessary than the culture slides supplied. This was checked experimentally by inoculating the same

suspension of an organism on glycerol nutrient agar in Petri dishes and in culture slides and incubating them at 28° C. in a saturated atmosphere. The results are given in Table 2.

All determinations were made at the end of 5 days of incubation at 28° C. Results for the slide cultures were determined microscopically, and were recorded as positive if growth occurred at any one of five points of inoculation. Results for the plate cultures refer to growth of individual inoculation points, which were determined by means of low power of a microscope (× 100).

This experiment showed that in the slide cultures out of 45 inoculations with 6 different organisms, only 13 or 29% grew; whereas on the poured plates of 144 inoculations of the same organisms, 125 or 86% grew. This indicates that some unfavorable environmental condition was present in the one case that was not present in the other. This may have been insufficient oxygen, since Sanford (1926) has shown that oxygen is a limiting factor both for germination and growth in *Actinomyces scabies*. Although the culture slides have a circuitous path for the entrance of air, the amount necessary for germination and growth may not be present.

TABLE 2.—*Comparison between growth of Nocardia inoculated on glycerol nutrient agar slide cultures and on poured plates.*

ORGANISM	SLIDES			PLATES		
	No. of Trials	Results		No. of Trials	Results	
		Pos.	Neg.		Pos.	Neg.
13–20	10	0	10	30	30	0
P. ruber	8	7	1	30	30	0
KLJ	9	5	4	30	25	5
P. minimus	6	1	5	18	15	3
N. polychromogenes	6	0	6	18	7	11
N. erythropolis	6	0	6	18	18	0
Totals.....	45	13	32	144	125	19

A method of growing the organisms on the surface of agar blocks, fully exposed to air, was then devised. This method was suggested by work of Ørskov (1922) on single-spore isolations of bacteria, and Jones (1940) on single-spore isolations of *Actinomyces*. Strips of agar, 1½ cm. x 4 cm. and 2 mm. thick were cut from poured plates. These strips were then placed on sterilized slides, and ringed with melted agar to cement them to the slide and to prevent drying on the exposed edges. Streak inoculations were then made on the surface of the medium. In

this way isolated organisms could be found for observation somewhere along the streak. In order to facilitate finding the same organism for successive observations, a small amount of wood charcoal was mixed with the suspension of inoculum (Jones, 1940). By mapping the organism to be studied in relation to particles of charcoal, and by keeping a record of the settings of the mechanical stage, no difficulty was experienced in finding the same organism for successive study. The cultures were then incubated in moist chambers at 28° C. or at room temperature. Observations and drawings were made as previously described.

The studies were carried through as many hours as was possible. The size and complexity of the growth usually determined how long a study could be continued. Observation became increasingly difficult as the colony grew, and studies were terminated when it became no longer practical to follow development. The time for this varied from organism to organism. In many cases the cultures were placed at 5° C. overnight, and studies continued the next day. This seemed to have little effect except to slow the rate of growth, and on subsequent culture at 28° C., the organisms continued to grow normally. Only one organism was encountered which would not tolerate this low temperature.

As many studies were made of an organism as seemed necessary to obtain a complete picture of the early growth. The number of studies of individual organisms varied from 4 to 27. The medium used was usually glycerol nutrient agar, but in some studies, Czapek's medium was used. No differences in development on the two media were found.

This method of study proved to be satisfactory for the early developmental studies. Little difficulty was experienced from air contaminants, as studies were usually terminated before they became a problem. The organisms because of their refractiveness were easily visible, when viewed on the surface of the agar strips. It was always necessary to keep the cultures at room temperature for a brief period immediately before making an observation in order to prevent fogging of the lenses of the objective.

The greatest disadvantage of this method is that even when great care is exercised in selecting an organism for study, the chances that it will grow are only one in three. From data kept on organisms that must be artificially fragmented by grinding the vegetative mycelium between slides in order to prepare the inoculum, it was found that of 101 individuals selected for study, only 33% grew. The strains which undergo fragmentation into short segments are more easily grown. Out of 338 trials of strains of this type growth in 188 (56%) was obtained. Two strains that were tried could not be grown at all from single isolated cells, by this method.

Another disadvantage of this technique is that it does not permit study of the colonial development beyond a degree of complexity which is reached rather early, so that other methods must be used to study the ultimate development of an organism.

In this manner, studies were made of the external developmental morphology of eighteen strains of *Nocardia*. Such details of external structure as time and manner of branching, orientation of branches,

occurrence and mechanism of fragmentation, and other habits of growth were delineated. Due to the differences in refractiveness of parts of the cytoplasm internal structure could sometimes be seen. No attempt was made to study or record this, as the magnification used did not permit detailed study of internal structure.

RESULTS

1. *Germination.*

In these studies two very different structures germinated; coccoid or bacillary cells, and portions of hyphae artificially torn from a mycelium. Strains which produce bacillary or coccoid elements, germinate by an elongation of the entire cell, which becomes a part of the hypha. Two structures similar to germ tubes formed at opposite ends of the coccoid cells in *P. agnosus* and *P. asteroides* var. *crateriformis*. In the mechanically produced hyphal fragments, growth proceeded by elongation of the hyphae. In some filaments only one end grew, whereas in others, growth was from both the ruptured and intact ends. Individual filaments within a strain differed in this respect although in a few strains growth occurred only from one end. It was found that, in general, longer hyphal fragments grow better than short ones. The shortest hyphal fragment observed to grow was 8 microns in length (Strain 13–3).

As was earlier indicated, germination of isolated elements was highly unpredictable. It was found practicable to select for growth studies only highly refractive cells or hyphal fragments, the latter being at least 10 microns in length. Even with the most careful selection only slightly more than half of the naturally produced fragments grew, and only one-third of those artificially produced. Although no statistical data were obtained, it was observed that the percentage of cells or fragments germinating increased with an increase of their numbers in a given field.

No special studies were made regarding the time which elapsed before germination began at a given temperature. The time of germination in Table 3 simply indicates when growth was first evident so that observations on development could begin. The actual time of germination would be earlier.

Growth, the first few hours, is one of elongation of a cell or hypha. Then primary branches are formed, or fragmentation occurs, depending on the organism. In forms which do not fragment, the initial hypha may elongate considerably before branching. Growth is accelerated gradually until it reaches a maximum rate, and then decreases slowly. However, peripheral hyphae grow for as long as nine days under the conditions provided in these studies.

2. *Branching.*

Branches are produced in *Nocardia* as simple lateral protuberances from a hypha. When first formed they are likely to have a smaller diameter than the mother hyphae and may have swollen tips. Branches, however, soon thicken to the diameter of the parent hyphae. They usually arise at right angles but may bend soon due to unequal inter-

calary growth. Branches probably arise from all surfaces of the cylindrical filaments, although this could not be determined because of their small diameter.

In sparsely branched forms (strain W-F), the hyphae are likely to remain straight. They never reach a very great length before breaking away at the base, or otherwise fragmenting. Secondary branches are not formed by organisms of this sort.

In the strains that branch more profusely (Strain 43–8), hyphae may reach a considerable length before fragmenting. They may curve and sometimes cross each other, but never become contorted. Branching proceeds in such a manner that the symmetry of the colony is radial. The older part of the original hypha produces branches first, and these generally grow to greater lengths than those produced by the younger parts. The time interval between primary branching and secondary branching is quite constant for a given strain. Tertiary branches are frequently produced. There seems to be no limit to the length of hyphae except as determined by fragmentation. Only monopodial branching was observed in the strains studied. In *P. ruber*, sometimes branches arise near the tip of a filament, suggesting dichotomy. (See Pl. 6, fig. G, 2 and 3).

In strains which do not fragment naturally, branches originate in the same manner as in the other types, but in general are more likely to be contorted and to overlap. There is a greater irregularity in the growth rate of successively produced branches. Primary branches may grow more slowly than others which arise much later. (Pl. 15, branch 4). In some strains the branches recurve and cross the parent hypha (Pl. 10, branch 4). In other strains such as *N. polychromogenes*, the branches are likely to produce the young colony, with the original hypha growing very little or not at all. In some instances branches are very closely spaced, forming a compact entanglement in the center of the young colony (Pl. 11). In others, branches are widely spaced (Pls. 13 and 14). In strain 13–3 (Pls. 16 and 17) the initial hyphae soon cease to elongate, and the branches proliferate very rapidly producing a more compact growth.

Other peculiarities of growth are typical of certain strains. Hyphae of some strains grow down into the agar (Pls. 16, 17 and 18), forming a sub-surface mycelium. In one strain, 20–6 (Pl. 18) a looping of hyphae is characteristic. Branches of some strains form acute bends, strains 13–10 (Pl. 11) and 7–7 (Pl. 12), whereas others curve gently.

3. *Fragmentation.*

In this paper "fragmentation" is used to describe the process of cell division in surface (or sub-surface) mycelium which results in the breaking up of mycelium into small rods (or coccoid cells). The term was used by Lachner-Sandoval (1898) to describe the same process in contrast to "segmentation" which leads to the formation of spores in aerial hyphae.

In these studies three very different arrangements of the fragmenting hyphae were observed. They are so pronounced as to be useful criteria in the separating of strains into groups.

The types of fragmentation are as follows:

Type 1. The division is preceded by a sharp bend in the hypha due to intercalary growth. Separation occurs at the apex of the bend and the ends of the two daughter cells grow out parallel to each other following division. Rarely, the ends grow across each other instead of growing parallel.

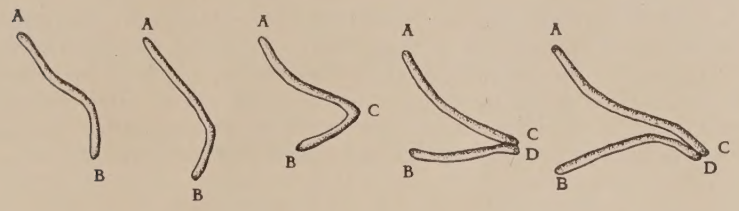


FIG. 1. Type 1 fragmentation. The filament A-B elongates and an acute bend is formed at C. Division occurs at C forming the ends C and D which grow out parallel to each other.

Type 2. Division may occur in a hypha in which there is little or no curvature. The two broken ends bend slightly away from each other and continue to grow. They may grow alongside each other, or in some cases bend more acutely and grow out in opposite directions.

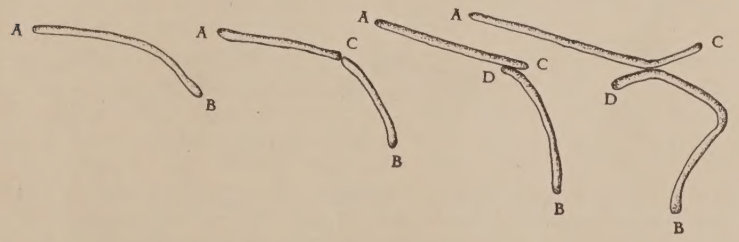


FIG. 2. Type 2 fragmentation. The filament A-B divides at C in a slightly curved portion of the hypha. This produces the ends C and D which bend away from each other and continue to grow.

Type 3. In some strains of *Nocardia*, division occurs primarily in the parent hypha near to or at the juncture of a branch. Following division the newly formed end of the filament bends slightly and continues to grow. The hypha with the branch attached may send out another branch at the place where separation occurred. If this happens the new branch bends so that it grows past the tip of the other hypha. Sometimes no new branch is formed at the place of separation.

This type is characteristic of the organisms in which division is delayed and mycelial development is consequently extensive. It is the most frequent type of fragmentation in some strains of *Nocardia*.

All three types may occur in one developing organism, in *P. ruber*, for example. In four strains (W-F, KLJ, B-B and *N. erythropolis*) only types 1 and 2 occur. This is correlated with the lack of prolific branching, and early division. The third type of fragmentation is associated with strains which form a more extensive mycelium, and in which division is delayed. Two strains (*P. agnosus* and *P. polychromogenes*) in which type 1 predominates but the other two types are present, are intermediate in this respect.

A kind of fragmentation may result from the death and disintegration of considerable sections of a mycelium. In all of the studies of *N. polychromogenes*, for example, at some time certain parts of the mycelium died, thus dividing the plant into two or more parts. In old material dead filaments are frequently found. The exact nature of this is unknown, but it may be due to autolysis of parts of hyphae.

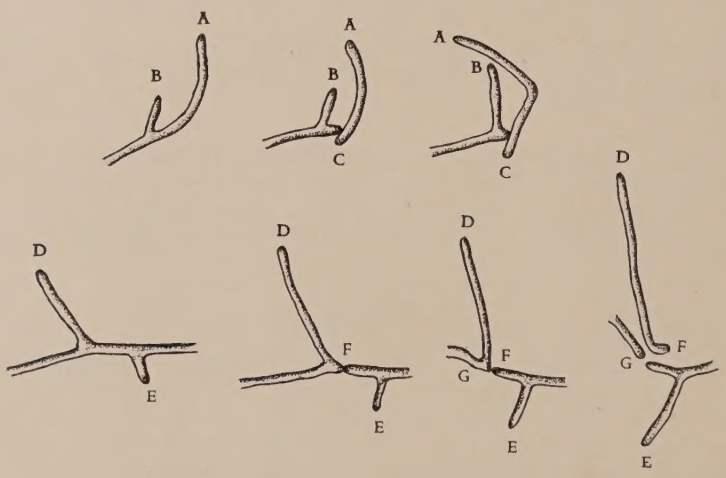


FIG. 3. Type 3 fragmentation. In the upper figures a division occurs at C near the base of branch B. In the lower figures the division occurs at F at the base of branch D. Later a second division occurs on the other side of the base of branch D at G, which separates the branch from the parent hypha.

4. Development of mycelium.

The degree and extent of mycelial development of the eighteen strains of *Nocardia* studied enables one to classify them into three groups:

Group I as represented by the first six organisms in Table 3 shows very early fragmentation which usually occurs before branching. In a few of the studies branching was not observed. Fragmentation of this group is characteristically type 1, (ranging from 40% to 70% depending on the strain). The early fragmentation results in the production of a limited amount of mycelium.

The occurrence of coccoid elements in old material is also associated with this group. No investigation was made as to their origin or

significance in this work. Krassilnikov (1938) states that both coccoid vegetative and resistant cells are characteristic of *Proactinomyces* (*Nocardia*).

Group II is characterized by a delay in fragmentation. This occurs only after 18 to 20 hours of incubation resulting in the production of a great deal more mycelium than in Group I. Group II includes strains 13–20, 43–8, *P. ruber* and probably *N. polychromogenes* and *P. asteroides* var. *crateriformis*. The latter two strains are known to fragment, but they did not do so under the conditions of the present study. Fragmentation of type 3 is most often present in this group, and type 1 is markedly uncommon. (See Table 3 for percentages of various types of fragmentation.)

Group III is characterized by a complete lack of fragmentation in the mycelium and is exemplified by the remainder of strains studied. In these organisms, so far as is known, no fragmentation of filament occurs. The colonial texture of these organisms is of two types. They are either waxy and can be separated by a needle into flakes (strains 21–3, 13–10, 13–16, 13–3 and 20–6) or have a cartilaginous texture (strains 18–2 and 7–7) and cannot be easily separated with the needle. No developmental morphological differences could be noted between these. Associated with strains in Group III is the very contorted growth habit of the mycelium which is not seen in Groups I and II.

There follows a description of the external morphological characters of the eighteen strains of *Nocardia* studied. The tabulation is arranged consecutively as are the plates to which they refer, beginning with strains that are bacterial in colonial characteristics, (i.e., *α Proactinomyces* of Umbreit (1939), to those that are more like *Streptomyces* in character (i.e., *β Proactinomyces* of Umbreit (1939)).

From four to twenty-seven separate developmental studies recorded as a series of *camera lucida* drawings were made for each of the eighteen strains. The data as presented are based on the over-all results as judged from repeated observations, so that a particular drawing on a plate may or may not strictly conform. The drawings were made from organisms growing on glycerol nutrient agar at 28° C. unless otherwise stated. Descriptions of colonial texture and pigmentation were always made from six months old cultures on glycerol nutrient agar.

Strain W-F (Plate 1, upper half, A to O). Seven studies were made of this strain, one of which is shown on Plate 1.

Growth begins after 8 hours of incubation at 28° C. from both ends of bacillary cells 1 to 3 × 0.7 microns. As the filament grows it bends because of unequal intercalary lengthening. Fragmentation generally occurs after 13 hours of incubation before any branches have formed. About 70% of the fragmentation is of type 1, the division occurring at the apex of the bend after the rod has reached a length of from 10–15 microns. (Pl. 1, figs. C-G.) About 30% of the fragmentation is type 2 in relatively straight hyphae. (Pl. 1, figs. G-H.) The daughter cells elongate, bend and again fragment. They may branch, but never profusely, and in some studies of this organism no branching was observed. When branches are formed they remain attached to the parent cell for a few hours, then break away at the point of attachment.

Their subsequent behavior is like that of other hyphae. The orientation of the fragments is such that a colony develops having radial symmetry. The rods become shorter, especially in the center of the young colony, and eventually coccoid cells are produced. The studies on Czapek's medium show the same progressive changes.

The mature colony has a soft, bacterial texture and an intracellular insoluble pigment (wood brown Ridgway Plate XL 17′ ′ ′) is produced.

P. agnosus (Plate 1, lower half, A′-U′). Sixteen studies were made of this strain, one of which is shown on Plate 1.

Growth starts after about 6 hours of incubation at 28° C. from both ends of a coccoid element 1 x 1.2 microns. The mycelium forms from germ tube-like hyphae on both sides of the coccoid cell. The hyphae curve somewhat and a division occurs at from 10–15 hours of incubation before or after a branch has formed. About 60% of the fragmentation is type 1 (Pl. 1, fig. O′, 9A and 9B), about 30% is type 2 (Pl. 1, fig. M′, 8A) and about 10% type 3. The coccoid fragment, due to its greater refractiveness, may remain visible for several hours. After fragmentation the ends of the divided hyphae may bend slightly and grow past each other, or they may grow parallel, if the pre-division bend is acute enough. As growth continues, the rods in the center become shorter, and coccoid elements are subsequently formed. However, some rods remain even after several months. Branching is always sparse with no extensive mycelial development. Secondary branches were never observed.

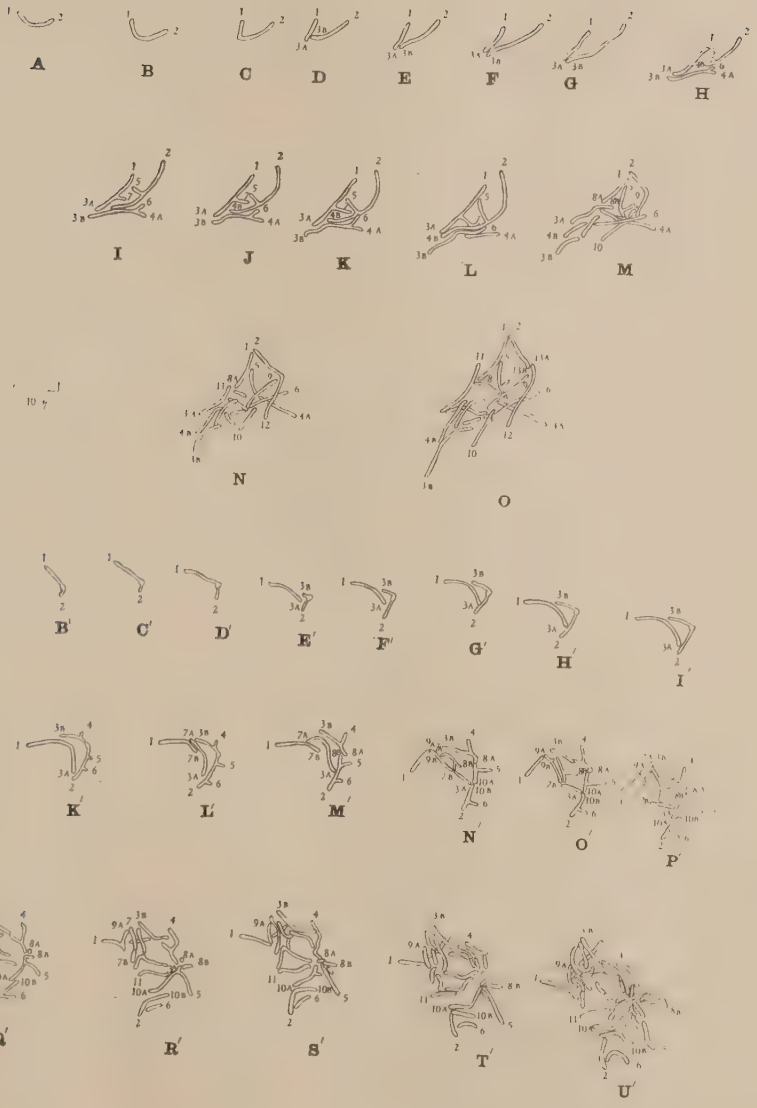
EXPLANATION OF PLATE I

Strain W-F. Glycerol nutrient agar; room temperature (circa 25° C). Study starts after 8 hours of incubation and drawings are at hourly intervals.

In fig. A growth has started after 8 hours of incubation. The rod continues to elongate and bends due to unequal intercalary growth. In fig. D after 13 hours of incubation fragmentation has occurred, without previous branching. Since the preceding bend was acute, the ends 3A and 3B grow parallel to each other in succeeding figures. The fragmentation in fig. H occurred where there was a curve in the filament. Ends 4A and 4B bend slightly and grow past each other. Branches 5 and 6 arise near the divided ends after 17 hours of incubation. The branches elongate, and hypha 1–3A produces a branch after 18 hours. Fragmentation occurs at the base of branch 6 in fig. L, so that branch 6 continues as the end of the original hypha. Fragmentation takes place rapidly from L to O in various hyphae.

P. agnosus. Glycerol nutrient agar; room temperature (circa 25° C). Study starts after 9½ hours of incubation and subsequent drawings are at ½-hour intervals. A 1½-hour interval at 5° C separates figs. R′ and S′.

In fig. A′ a germ tube-like extension has grown from one side of the coccoid cell. This elongates and in fig. B′, ½ hour later, another starts on the opposite side. These grow out and in fig. E′ a fragmentation has occurred close to the coccoid cell, which is still visible. Two hyphae are produced by this fragmentation. These grow and in fig. K′ after 13 hours of incubation three branches are produced by hypha 2–3B. A fragmentation occurs in hypha 1–3A in fig. L′, the newly formed ends 7A and 7B bend slightly and continue to grow. Hypha 1–7B bends and fragments at the apex of the bend in fig. N′, the ends 9A and 9B grow out parallel to each other in figs. O′, P′, Q′, etc. Branches are produced in greatest number in figs. N′, O′, and P′ (17–18 hours), and subsequently fragment at their bases in figs. R′ and S′. Subsequent development consists in elongation and fragmentation of hyphae.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Colonies present a soft, mucoid texture, with an insoluble (light ocraceous buff Ridgway Plate XV 15'd) intracellular pigment.

Strain KLJ (Plate 2, upper half, A-R). Fourteen studies were made of this strain, one of which is shown on Plate 2.

Growth begins after 8 hours of incubation at 28° C. from both ends of rods 3 to 5 × 0.7 microns. After about 13 hours of incubation when the cell is 10–15 microns in length, it fragments. About 50% of the fragmentation is type 1 (Pl. 2, fig. D, 3A and 3B) and about 50% type 2 (Pl. 2, fig. J, 7). The hyphae may or may not branch. When branches are produced they may also fragment by either of the two methods and they eventually separate from the parent hypha. The hyphae in the center of the colony fragment into shorter and shorter rods, and these may finally produce coccoid elements. The orientation of the fragments is such that a circular colony results. Two studies on Czapek's medium show the same development as that described.

The colonies are pasty in texture and an insoluble, intracellular pigment (light vinaceous-cinnamon, Ridgway Plate XXIX 13'' d) is produced.

Strain B-B. (Plate 2, lower half A' to O'). Six studies were made of this strain, one of which is shown on Plate 2.

Growth begins after 10 hours of incubation at 28° C. from both ends of rods 6 to 10 × 0.9 microns. A hypha grows to about 20 microns in length after about 14 hours of incubation and fragmentation occurs, without previous branching. Again about 50% of the fragmentation is type 1 (Pl. 2, fig. C' and D'), and 50% type 2 (Pl. 2, figs. K', L', M', 7–13). In a few divisions the bending is somewhat intermediate so

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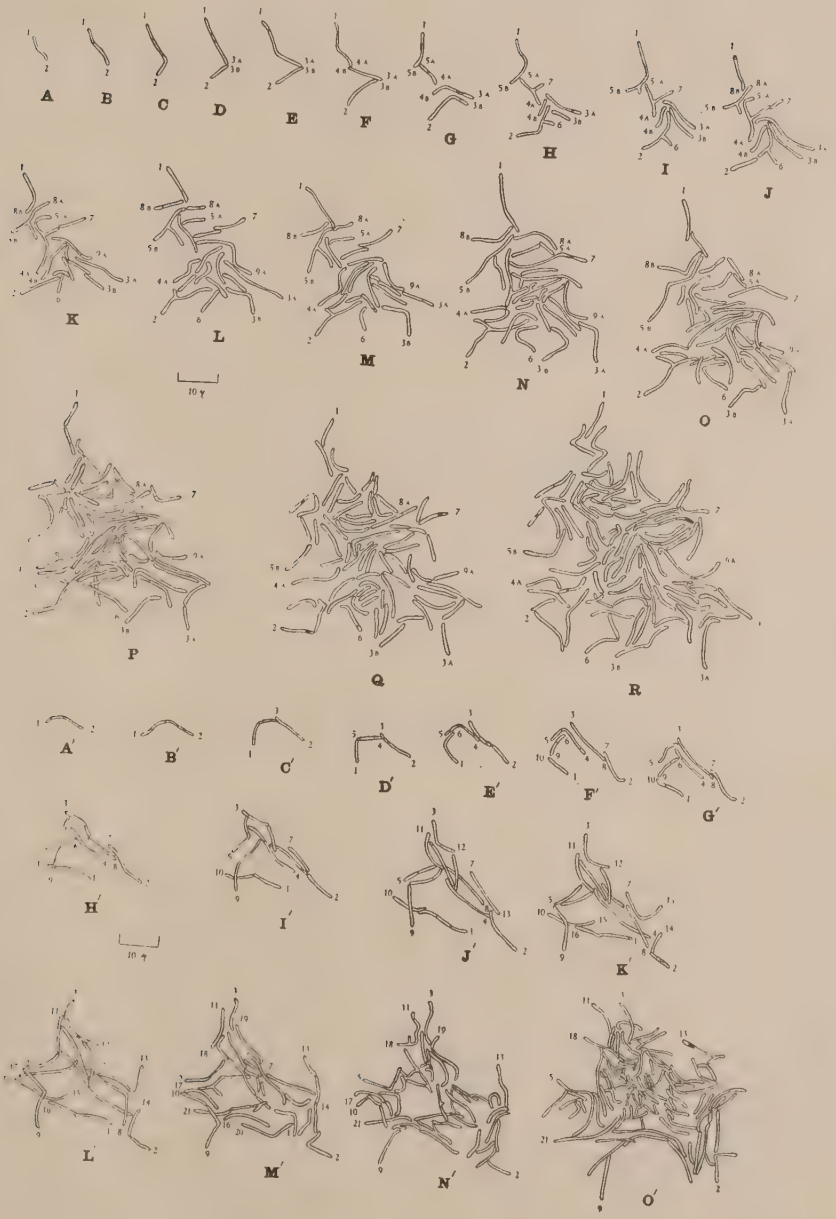
EXPLANATION OF PLATE 2

Strain KLJ. Glycerol nutrient agar; 28° C. Study begins after 10½ hours of incubation and drawings are made at hourly intervals. An 11-hour interval at 5° C separates figs. Q and R.

The rod elongates from figs. A to C. A bend is produced in fig. C at the apex of which fragmentation occurs in fig. D. The ends 3A and 3B grow out parallel in figs. F and G. The fragmentation in fig. F is produced at a slight curvature, but the ends 4A and 4B finally grow in the same direction as shown in figs H through K. The first branches are produced in fig. H after 17 hours of incubation. Branch 7 fragments in fig. J and breaks away from the mother hypha at fig. L. Branch 6 fragments at its base at fig. K. Following fig. K, fragmentation occurs in various hyphae, in either straight (fig. M, 3A) or more frequently at curved regions of hyphae. Short branches may be formed by some hyphae, but mycelial development is limited. The hyphae in the center of the young colony begin to get shorter at fig. P; the peripheral hyphae continue to grow and fragment. Orientation is such that the colony becomes radially symmetrical.

Strain B-B. Glycerol nutrient agar; room temperature (circa 25° C). Study begins after 10 hours of incubation and drawings are at hourly intervals. A 14-hour interval at 5° C separates figs. K' and L'.

The rod elongates, curves and fragmentation occurs in fig. C' after 12 hours of incubation. Another occurs in fig. D', and the ends 3 and 4, 5 and 6 respectively curve slightly and grow past each other. Hypha 1–6 curves and a division occurs which results in the ends 9 and 10. They cross and grow. The colony enlarges considerably by the elongation, bending and fragmenting of hyphae. The first branch is produced in fig. M'. Branches are infrequently produced in this study. The rods begin to get shorter in the center of the colony in figs. N' and O'. The peripheral hyphae elongate, bend and fragment, and occasionally branch. The orientation of hyphae produces a circular young colony.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that the two ends grow across each other (Pl. 2, fig. H', 9 and 10). Branches may develop on older hyphae, but seldom on young ones. The center of the young colony consists of smaller and smaller rods, and coccoid elements may be produced. The peripheral hyphae continue to grow and fragment.

The colony has a soft bacterial texture and an intracellular insoluble pigment (light vinaceous-cinnamon, Ridgway Pl. XXIX 13' ' d) is produced.

Nocardia erythropolis (Plate 3, upper half A to R). Eleven studies were made of this strain, one of which is shown on Plate 3.

Growth begins after 11 hours of incubation at 28° C. from both ends of rods 5 to 15 × 0.8 microns. An initial mycelium is usually not produced as fragmentation precedes branching. However, sometimes as many as three short branches may be present before fragmentation. The first fragmentation usually occurs after 14 hours of incubation and may be multiple. About 60% of the fragmentation is type 2 (Pl. 3, fig. I, 5A and 5B), and about 40% is type 1. The reorientation of the fragmented ends depends on the amount of bending preceding separation. This organism differs from the former one chiefly in that the hyphae are longer before division occurs. The hyphae in the center of the young colony continue to fragment; however, no coccoid cells are produced.

The colonial texture is pasty and an intracellular insoluble pigment (light pinkish cinnamon, Ridgway Pl. XXIX 15' ' d) is produced.

P. polychromogenes (Plate 3, lower half, A'-S'). Nine studies were made of this strain, one of which is shown on Plate 3.

Growth begins after 11 hours of incubation at 28° C. from both ends of rods, 1 to 3 × 0.4 microns. A branch may be produced and then fragmentation occurs, or the hypha may fragment without branch-

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EXPLANATION OF PLATE 3

N. erythropolis. Glycerol nutrient agar; 28° C. Study begins after 10 hours of incubation and drawings are at hourly intervals.

From figs. A to E the rod elongates and bends. At the apex of the bend in fig. F a branch, 3, is produced. The hyphae continue to elongate and in fig. I multiple fragmentation has occurred, resulting in the production of three hyphae. The ends of the newly produced hyphae 4A and 4B; 5A and 5B respectively, curve and grow past each other. During the next hour two more fragmentations occur in fig. J. Branch 3 fragments at its base in fig. M. Only short branches are subsequently formed. The hyphae fragment rapidly from fig. M through fig. R. The hyphae become short in the center of the developing colony while the peripheral ones continue to elongate and divide.

P. polychromogenes (Vallée) Jensen. Glycerol nutrient agar; 28° C. Study begins after 10 hours of incubation and drawings are at hourly intervals. An 11-hour interval at 5° C separates figs. O' and P'.

The rod elongates and in fig. C' a branch is produced. The first fragmentation occurs in fig. E' at the base of branch 3 after 14 hours of incubation. The two hyphae 1–3 and 2–4 produce branches again in fig. F'. A fragmentation at the base of branch 6 occurs in fig. G'. Other branches are produced and fragmentation is frequent at their bases. The hyphae continue to grow and fragment and in the center of the young colony the rods become shorter, while the peripheral hyphae continue to grow.


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ing. Fragmentation begins after about 14 hours of incubation, and the first branch is usually produced at 13 hours. About 30% of the fragmentation is type 3 (Pl. 3, fig. E', 3 and 4), about 40% type 1, and 30% type 2 (Pl. 3, fig. J', 10). The orientation of the ends of the newly divided hyphae depends on the amount of curvature previously produced. The central hyphae continue to fragment, and the peripheral ones continue to grow out.

The colonies have a pasty texture, and an intracellular insoluble pigment (rose doree Ridgway Plate I 3 b) is produced.

Strain 13–20 (Plate 4). Sixteen studies were made of this strain, one of which is shown on Plate 4.

Growth begins after 11 hours of incubation at 28° C. from both ends of rods 1 to 2 × 0.8 microns. Branching always occurs before fragmentation; from 4 to 14 branches may be produced before fragmentation occurs. In some cases secondary branching has occurred before fragmentation can be observed. Fragmentation is of all three types; about 66% is type 3, 28% type 2, and 6% type 1. Primary branches are produced from 11 to 14 hours of incubation, and secondary ones arise 4 to 7 hours thereafter. In most instances fragmentation of the primary mycelium has occurred before secondary branches are formed. The original mycelium remains the principal one. The ends of hyphae tend to grow alongside each other on meeting, although they occasionally cross. The hyphae in the center of the young colony fragment rapidly, while peripheral ones continue to grow and branch.

Colonial texture is pasty and an insoluble intracellular pigment (light salmon-orange Ridgway Plate II, 11d) is produced.

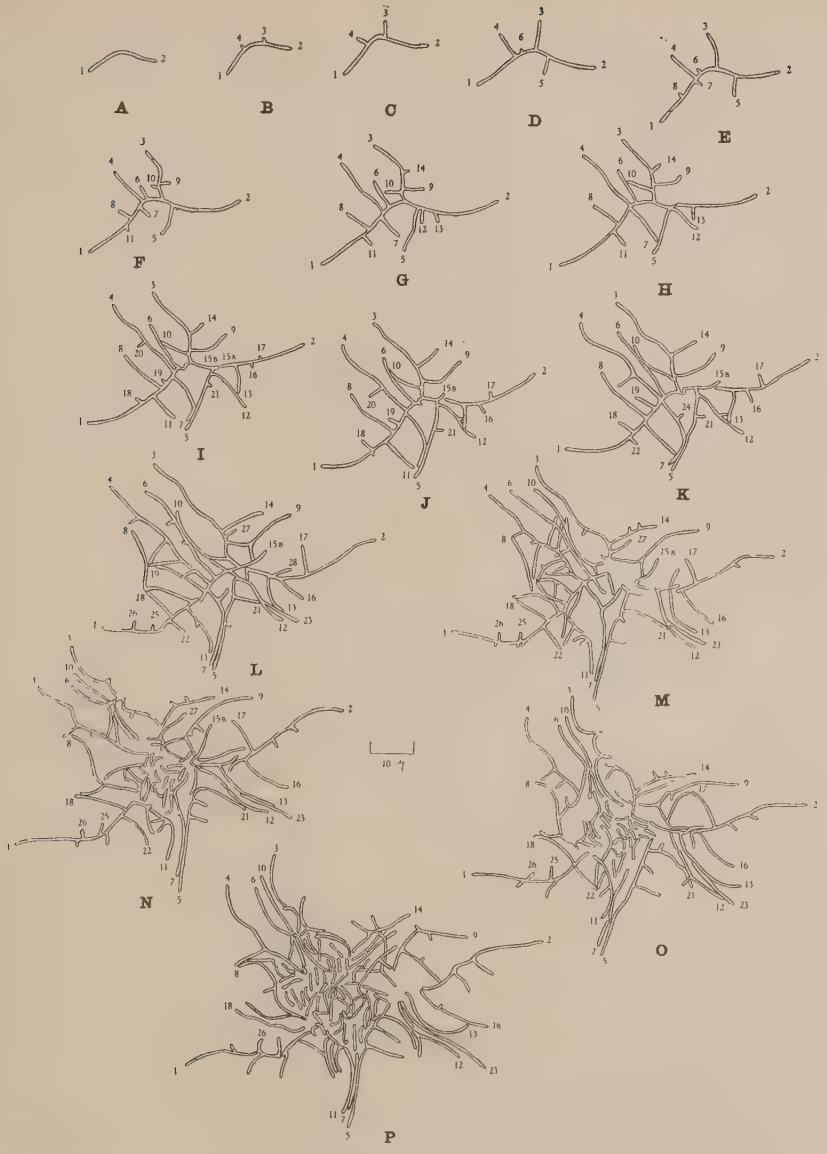
Strain 43–8 (Plate 5). Four studies were made of this strain, one of which is shown on Plate 5.

Growth begins after ten hours of incubation at 28° C. from both ends of rods 3 to 8 × 0.7 microns. Branches are always produced before fragmentation occurs. Primary branching takes place after about 12 hours of incubation and secondary branches are produced 7 hours later. About 50% of the fragmentation is type 3 (Pl. 5, fig. J, 14 and 15), about 45% type 2 (Pl. 5, fig. I, 12 and 13) and only 5% is type 1 (Pl. 5, fig. K, 17 and 18). The peripheral hyphae are longer than the central ones; they continue to grow and branch, whereas the central ones fragment into smaller and smaller elements. Eventually short rods about 1.5 microns in length are produced.

The colonial texture is pasty, and an insoluble intracellular pigment (salmon buff, Ridgway Plate XIV 11' d) is produced.

P. ruber (Plate 6). Twenty-seven studies were made of this strain, one of which is shown on Plate 6.

Growth begins after 10 hours of incubation at 28° C. from both ends of rods from 3 to 5 × 0.7 microns. Primary branches are produced at 14 hours and secondary ones about 4 hours later. In most studies secondary branches are produced before there is any fragmentation. Initial fragmentation which separates the mycelium into rather large units, occurs after about 20 hours of incubation and is always type 3. (Plate 6, fig. K, 20.) About 65% of the fragmentation is type 3; 30%



Strain 13–20. Glycerol nutrient agar; 28° C. Study begins after 11 hours of incubation and drawings are at hourly intervals. An 11-hour interval at 5° C separates figs. N and O.

The slightly curved rod continues to elongate and two branches are produced in fig. B after 12 hours of incubation. Other branches are produced in rapid succession. A fragmentation of the parent hypha occurs in fig. H which separates the mycelium into two parts. These two ends, 15A and 15B, curve and continue to grow. The branches elongate rapidly, and secondary branching begins in fig. I (branch 20). The branches grow parallel to each other when they meet. The parent hypha again undergoes fragmentation in fig. L between branches 19 and 8. Following this fragmentation, other filaments break up rapidly, especially in the center of the young colony, as shown in figs. M to P. The peripheral hyphae continue to grow and branch, while the hyphae in the center become shorter and shorter rods.

is type 2, and only 5% type 1. Fragmentation proceeds faster in the center of the colony with the production of smaller and smaller bacillary elements. The peripheral hyphae continue to grow, branch, and fragment. Orientation is such that the colony is essentially circular. Nine studies on Czapek's and three on Bennett's media show the same development.

The colonial texture is pasty and an intracellular insoluble pigment (flame scarlet, Ridgway Plate II 9) is produced.

N. polychromogenes (Plates 7 and 8). Six studies were made of this strain, one of which is shown on Plates 7 and 8.

Growth begins after about 14 hours of incubation at 28° C. from one or both ends of rods 5 to 15 × 0.7 microns. The growth is slow, and primary branching is delayed from 21 to 50 hours after inoculation, at which time the fragment has elongated to about 20 microns. Secondary branches are produced about 14 hours later. The branches grow out at right angles and are curved, but not contorted. They do not as a rule cross each other, but bend and grow alongside each other. In all studies portions of the original filament died (Pl. 7, fig. G) producing two or more mycelia after about 40 hours of incubation. In some studies the entire original hyphal fragment died, and the resulting colony developed from branches that had been previously produced. Branching always is delayed and growth is slow. The branches grow very long and a spreading diffuse growth results. The organism fragments, but only after at least five days, when it is impossible to study it.

The colonial texture is dry and thin and an insoluble intracellular pigment (coral pink, Ridgway Plate XIII d) is produced.

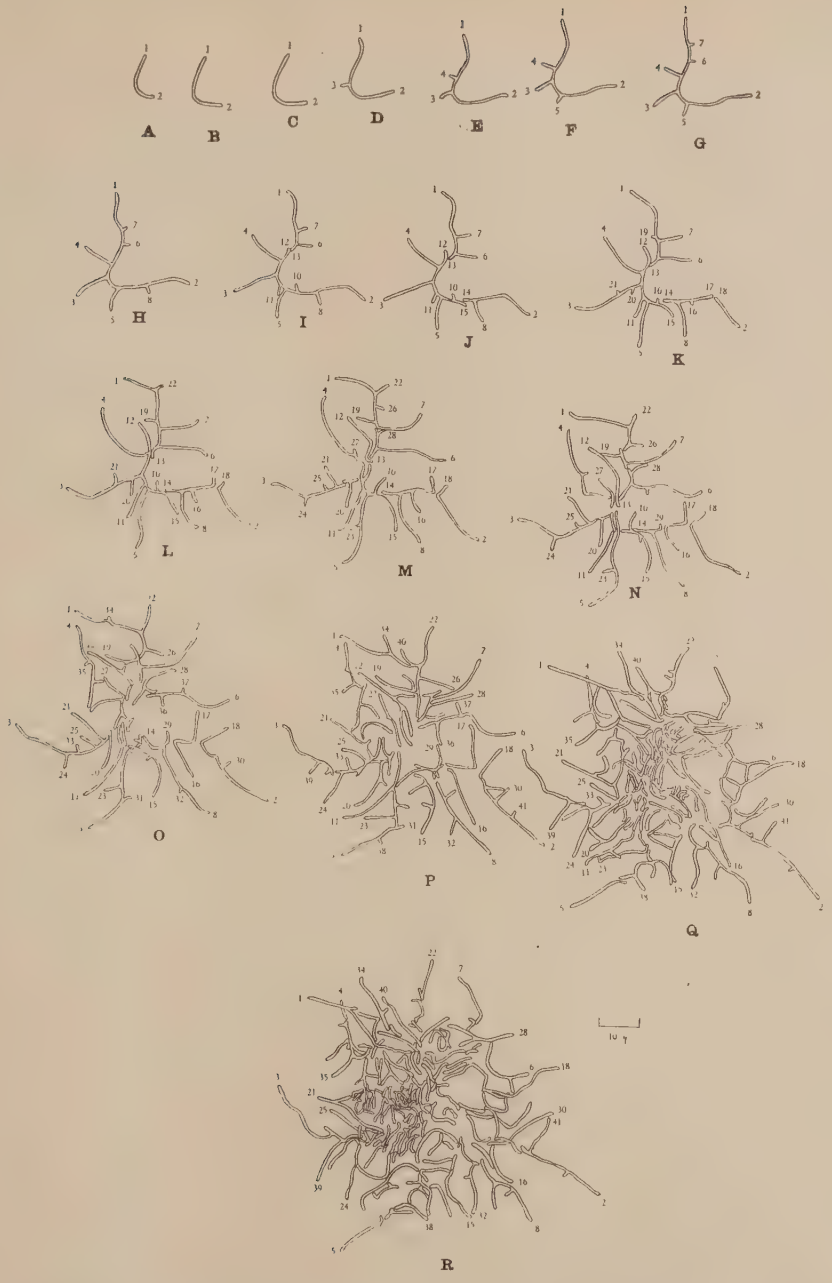
P. asteroides var. *crateriformis* (Plate 9). Four studies were made of this strain, one of which is shown on Plate 9.

Growth begins after about 10 hours of incubation at 28° C. from a small coccoid element about 0.4 micron in diameter. The mycelium forms from germ tube-like hyphae on two sides of the germinating element. Growth is slow and primary branching occurs after about 24 hours of incubation. The branches grow slowly and are short. They curve and sometimes form sharp bends, however, fragmentation is delayed until after four days of incubation, when details are obscure. As the organism becomes older the branches begin to bend more acutely and they become contorted. Sometimes aerial hyphae develop.

The colonial growth is thin, and can be removed easily from the surface, although there is sub-surface growth. An intracellular insoluble pigment (cinnamon buff, Ridgway Plate XXIX 17 ' ' b) is produced.

Strain 21–3 (Plate 10). Six studies were made of this strain, one of which is shown on Plate 10.

Growth begins after about 10 hours of incubation at 28° C. from only one end of an artificially produced hyphal fragment 10 to 30 × 0.9 microns. This organism is very difficult to grow under the conditions provided. It branches very sparsely at the end of about 16 hours of incubation. The hyphae are contorted and the branches overlap the parent filament. This organism tolerates storage at 5° C. very poorly. Growth is delayed on returning to 28° C. and may not be resumed.



Strain 43–8. Glycerol nutrient agar; room temperature (circa 25° C). Study begins after 10 hours of incubation and drawings were made at hourly intervals. A 16-hour interval at 5° C separates figs. O and P.

The curved rod continues to elongate and branches initially in fig D after 13 hours of incubation. Other branches are produced in rapid succession. The first fragmentation occurs in fig. I (12 and 13) after 18 hours of incubation. The ends of the newly formed hyphae subsequently bend and grow. Another fragmentation in fig. K produces ends 17 and 18, which grow parallel. Fragmentation becomes general in the center of the young colony in fig. O. The central hyphae become shorter while the peripheral ones continue to grow and branch.

No extensive mycelium was formed in any of the studies made. Fragmentation does not occur. This organism apparently grows better on Czapek's medium than on glycerol nutrient agar.

The mature colony has a waxy texture and can be easily separated with the inoculating needle into flakes. An intracellular pigment (apricot orange, Ridgway Plate XIV 11') and a brown soluble pigment are produced.

Strain 13–10 (Plate 11). Seven studies were made of this strain, one of which is shown on Plate 11.

Growth begins after about 9 hours of incubation at 28° C. from one or both ends of an artificially produced hyphal fragment 10 to 40 × 1 microns. Primary branches are produced after 11 hours of incubation and secondary ones about 6 hours thereafter. Contorted branches are profusely produced so that the center of the young colony becomes a plexus of filaments. No fragmentation of filaments occurs, but in old material many segments of filaments are empty, and others are highly cytoplasmic. Two studies of Czapek's show essentially the same growth pattern.

The colonial growth has a waxy texture and can be removed with a needle into flakes. An insoluble intracellular pigment (light buff, Ridgway Plate XV 17' F) is produced.

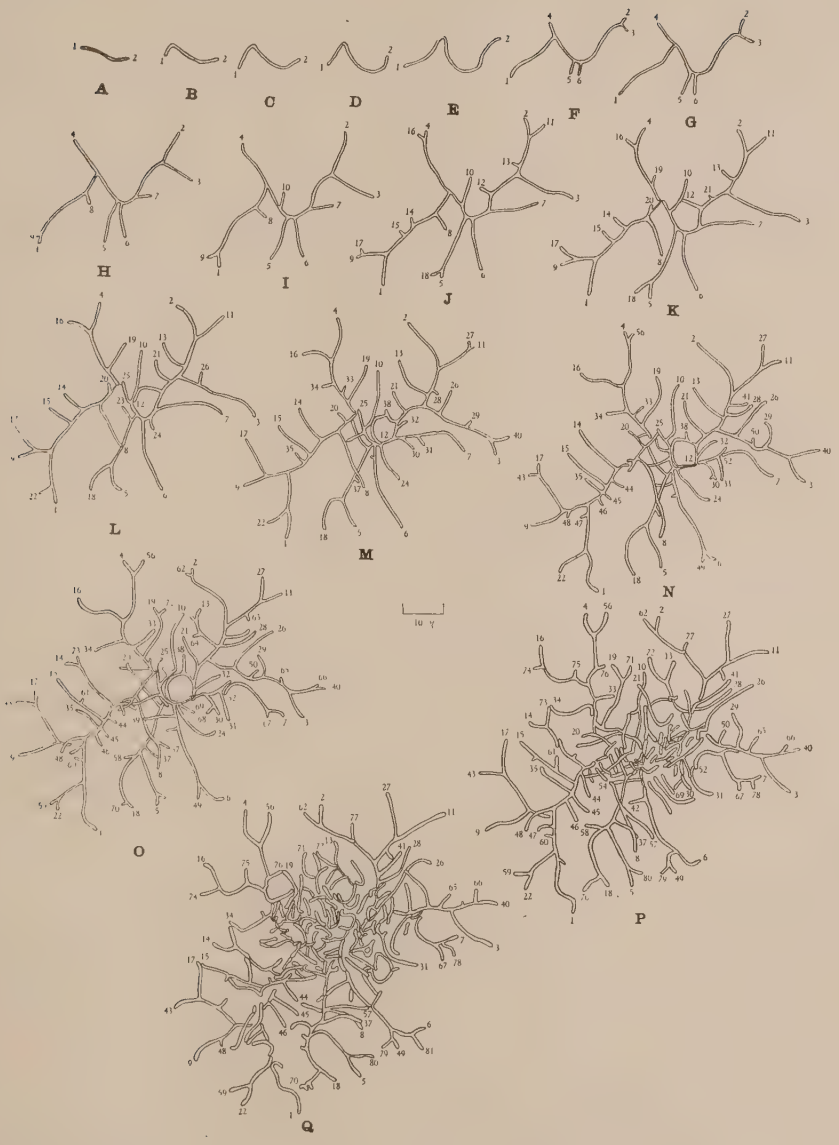
Strain 7–7 (Plate 12). Five studies were made of this strain, one of which is shown on Plate 12.

Growth begins after about 10 hours of incubation at 28° C. from one or both ends of an artificially produced hyphal fragment 10 to 30 × 0.7 microns. Growth is rapid and branching profuse. Primary branches are produced after about 12 hours of incubation and secondary ones 8 hours later. Branches arise at right and acute angles at both straight and curved places in the hyphae. They are contorted and many acute bends are formed. (Pl. 12, fig. L, 8 and 11.) The branches cross freely thus forming a plexus of branches in the center of the colony. However, in time, branching is also profuse in the peripheral hyphae. No fragmentation occurs.

The colonies have a tough cartilaginous texture and a soluble pigment (mummy brown, Ridgway Plate XV 17' m) is produced.

Strain 18–2 (Plates 13 and 14). Fourteen studies were made of this strain, one of which is shown on Plates 13 and 14.

Growth begins after about 11 hours of incubation at 28° C. from only one end of an artificially produced hyphal fragment 20 to 50 × 0.7 microns. Primary branches are produced after about 15 hours and secondary ones 6–7 hours later. They are widely spaced so that the colony is not compact when young. Some branches are contorted (Pl. 14, fig. R, 3), others are not. The primary branches are likely to grow faster than the parent hyphae, and become the principal part of the plant. The filaments tend to become more contorted as the colony develops. The ends of some hyphae (Pl. 14, fig. R, 4) grow down into the agar. No fragmentation occurs. Four studies on Czapek's medium show no essential differences from those on glycerol nutrient agar.



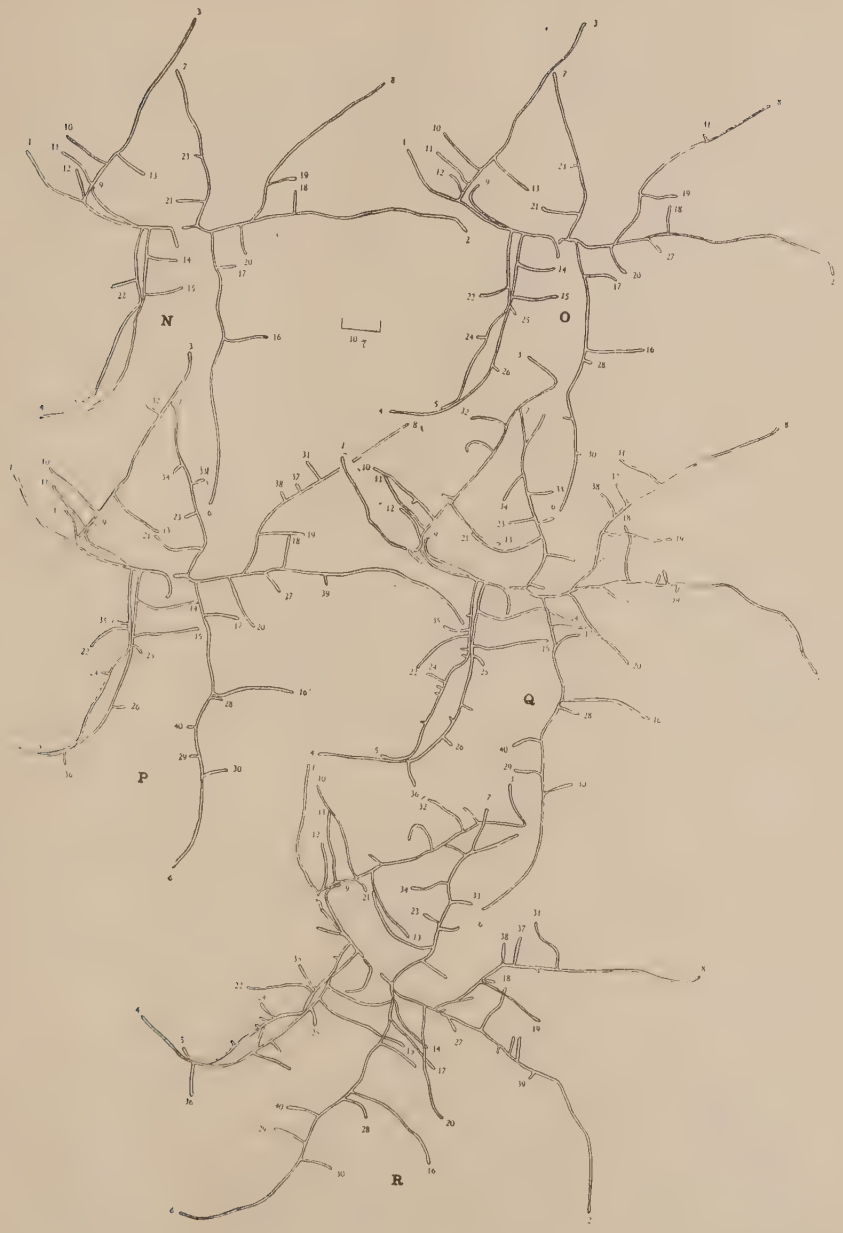
P. ruber (Casabó) Bald. Czapek's agar; 28° C. Study begins after 10 hours of incubation and drawings are at hourly intervals.

The rod in figs. A, B, C, and D elongates and curves. Branches are first produced in fig. F after 15 hours of incubation. Other branches arise rapidly from fig. H through fig. M. The first secondary branch, 26, arises in fig. L after 21 hours of incubation. First fragmentation occurs at the juncture of branches 1 and 4 in fig. K. This results in three separate mycelia being produced. The branches arise so close to the ends of other branches that dichotomy is simulated; 9 and 1 in fig. H, 9 and 17 in fig. J, for example. Branching continues, and general fragmentation in the center begins in fig. O after 24 hours of incubation. The center fragments rapidly; only one hour separates figs. O and P. The peripheral hyphae continue to grow and branch.

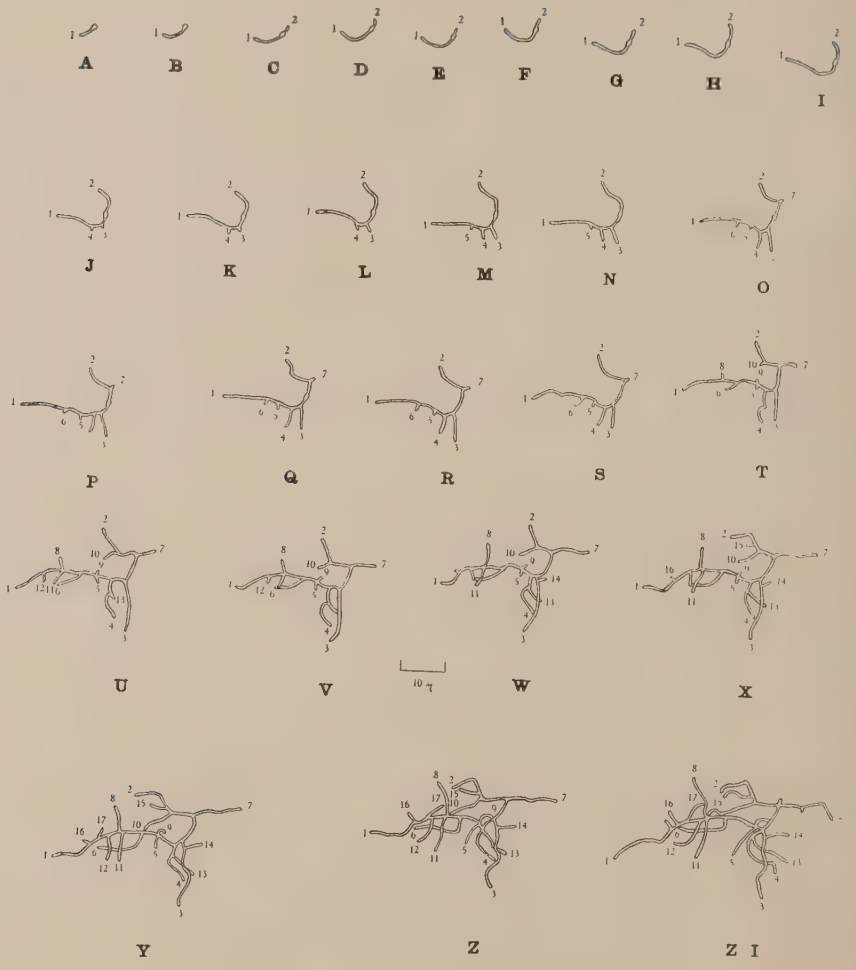
PLATES 7 AND 8

N. polychromogenes. Glycerol nutrient agar; 28° C. Study begins after 30 hours of incubation. Drawings are at from two to six hour intervals. A 10-hour interval at 5° C separates figs. B and C; 10 hours at circa 25° C separates figs. I and J; 15 hours at 5° C separates Q and R.

The hyphal fragment elongates in figs. A, B, C, and D. In fig. E after 40 hours of incubation a branch is formed. Other branches are formed in fig. F



which is two hours later than fig. E. A separation of the parent mycelium into two parts occurs in fig. G, which is after 44 hours of incubation. Following this the ends of the hyphae continue to grow. The branches grow out and do not cross each other. They remain relatively straight. Secondary branches arise in fig. I after 64 hours of incubation. This organism grows slowly, and fragmentation can be seen to begin in the center of the colony in fig. R, which is the result of 90 hours of incubation.

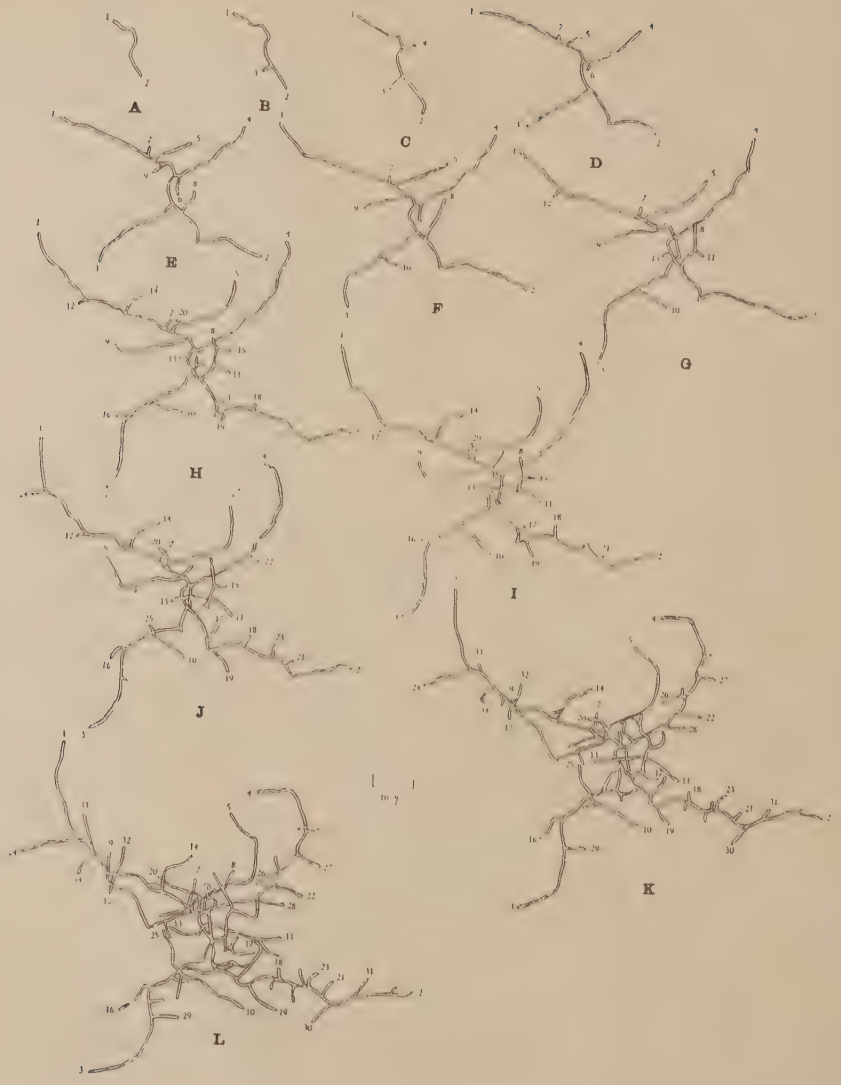


P. asteroides (Eppinger) Bald var. *crateriformis* Bald. Glycerol nutrient agar; room temperature (circa 25° C). Drawings are at 2-hour intervals. A 10-hour interval at 5° C separates figs. O and P; and 9 hours at 5° C separates figs. X and Y.

A germ tube-like hypha is formed from the germinated coccoid cell in figs. A and B. A second such structure is produced in fig. C. These grow out very slowly, and primary branches are produced in fig. J after 32 hours of incubation. Other short branches are produced in figs. M and O. Growth is very slow. The first secondary branch, 13 in fig. U, is produced after 46 hours of incubation. In fig. Z there is considerable shortening of the space between branches 4 and 5 which may indicate a beginning of fragmentation. The figure at ZI indicates the extent of mycelial development at the end of 53 hours of incubation. It is sparse; the branches remain short.

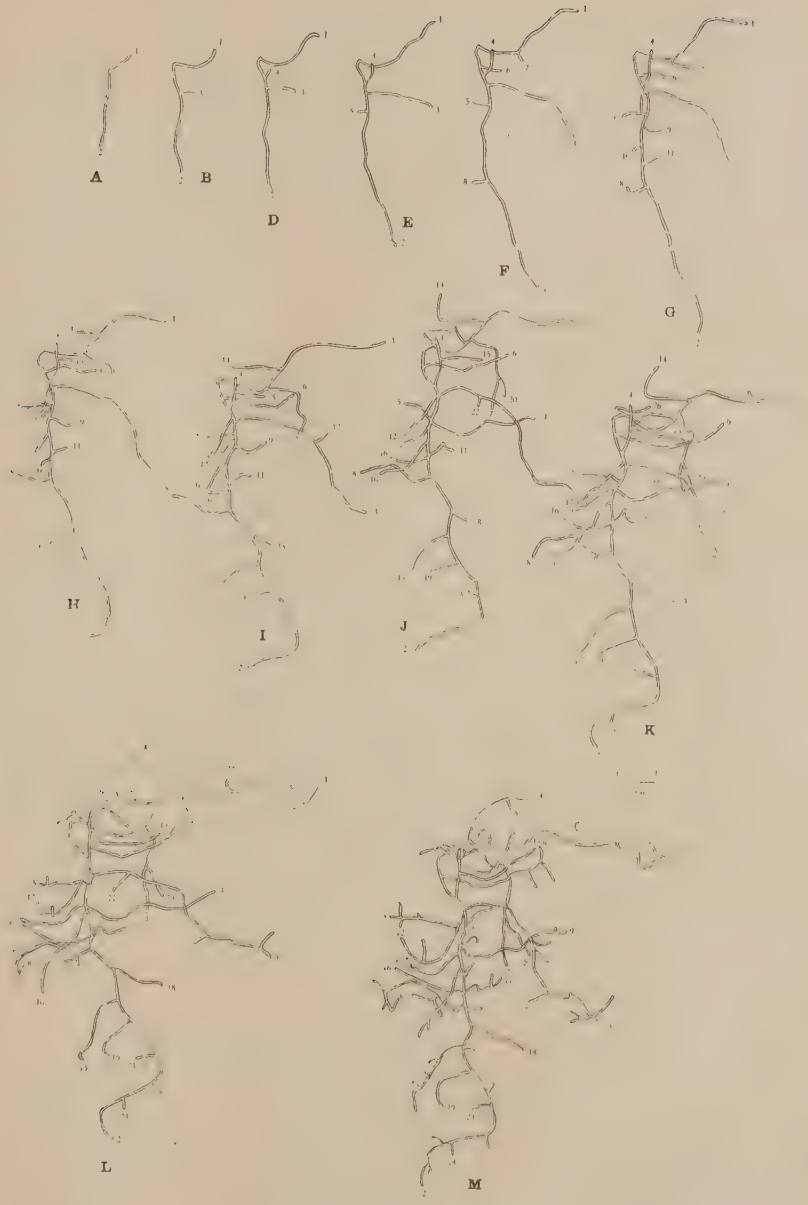
Strain 21–3. Czapek's agar; room temperature (circa 25° C). Study begins after 11 hours of incubation. Drawings are at 1 and 2 hour intervals. A 9-hour interval at 5° C. separates figs. M and N.

Growth begins from a curved hypha in figs. A, B, and C. Only end 2 grows. It curves and crosses the parent hypha in fig. E. Primary branches are produced in fig. H after 19 hours of incubation. Branch 4 grows across the parent hypha and 2 crosses 3. There is little change in figs. N. O. and P, due to the slowness with which growth is resumed after storage at 5° C in this organism. Then in fig. Q another branch forms. This organism branches sparsely and grows slowly under the conditions provided.



Strain 13–10. Glycerol nutrient agar; 28° C. Study begins after 9 hours of incubation. A 12-hour interval at 5° C separates figs. J and K. Drawings are at hourly intervals.

Growth begins by the elongation of the curved hypha in figs. A and B. A primary branch is formed in fig. B after 10 hours of incubation. It grows out rapidly and branch 4 produced after 11 hours of incubation also grows quite rapidly. These two branches soon reach the length of the parent hypha in fig. G, and help form the pattern of the young colony. Other branches are produced; the first secondary branch is in fig. E after 13 hours. Branching becomes more and more profuse and the center of the young colony becomes a complex of hyphae in fig. L after 24 hours of incubation. The numerous short branches and the acute bends such as in number 2 in fig. D, characterize this organism.



Strain 7–7. Glycerol nutrient agar; 28° C. Study begins after 10 hours of incubation. A 2-hour interval at 5° separates figs. J and K. Drawings are at hourly intervals.

The long hyphal fragment in fig. A branches in fig. B after 11 hours of incubation. The first secondary branch, 15, is formed in fig. H after 16 hours. The parent hypha continues to elongate; branches are produced profusely in the center of the young colony. The branches cross freely, 4 in fig. E, 12 in fig. H, etc., and are contorted. Branching increases as the colony develops and in fig. M after 22 hours of incubation many branches have arisen.

The colonial texture is tough and cartilaginous and an intracellular soluble pigment (slate color, Ridgway Pl. LII carbon gray K) is produced.

Strain 13–16 (Plate 15). Ten studies were made of this strain, one of which is shown on Plate 15.

Growth begins after an incubation period of about 10 hours at 28° C. from both ends of an artificially produced hyphal fragment 10 to 50 × 1.0 microns. Primary branching occurs after 11 to 17 hours of incubation and secondary ones develop about 4 hours later. The hyphae are contorted and the numerous short branches go out from the main filament producing a dendritic pattern. The branches may bend acutely, overlap and cross freely. The original filament remains the main axis of growth, and no fragmentation occurs.

The colonial texture is waxy and can be removed with a needle in flakes. An intracellular insoluble pigment (cinnamon-buff, Ridgway, Pl. XXXIX 17 ' ' b) is produced.

Strain 13–3 (Plates 16 and 17). Eleven studies were made of this strain, one of which is shown on Plates 16 and 17.

Growth begins after about 10 hours of incubation at 28° C. usually from only one end of an artificially produced fragment 10 to 30 × 0.8 microns. In a few cases the original filament did not elongate, but produced branches which formed the colony. Primary branching occurs after about 13 hours of incubation and secondary ones arise about 9 hours later. The main hypha ceases to grow and branches are produced profusely, resulting in a radially symmetrical young colony. Hyphae commonly grow down into the agar. Branches sometimes cross each other, however, many times they bend sharply away after touching another filament. (Pl. 17, fig. T, 19 and 35.) The young colony is diffuse due to the wide spacing of branches. No fragmentation occurs. Growth on Czapek's medium shows no essential differences from that on glycerol nutrient agar.

The colonial texture is waxy and an insoluble pigment (light pinkish cinnamon, Ridgway Pl. XXIX 15 ' ' d) is produced in the center of colonies, while the periphery remains unpigmented.

Strain 20–6 (Plate 18). Thirteen studies were made of this strain, one of which is shown on Plate 18.

Growth begins after about 8 hours of incubation at 28° C. from both ends of an artificially produced hyphal fragment 20 to 50 × 0.8 microns. Primary branches are produced in from 10 to 17 hours of incubation and secondary ones arise 6 to 8 hours later. The branches are compactly arranged and strongly contorted. They tend to form loops and grow down into the agar. No fragmentation occurs. Growth on Czapek's medium show no essential differences from that on glycerol nutrient agar.

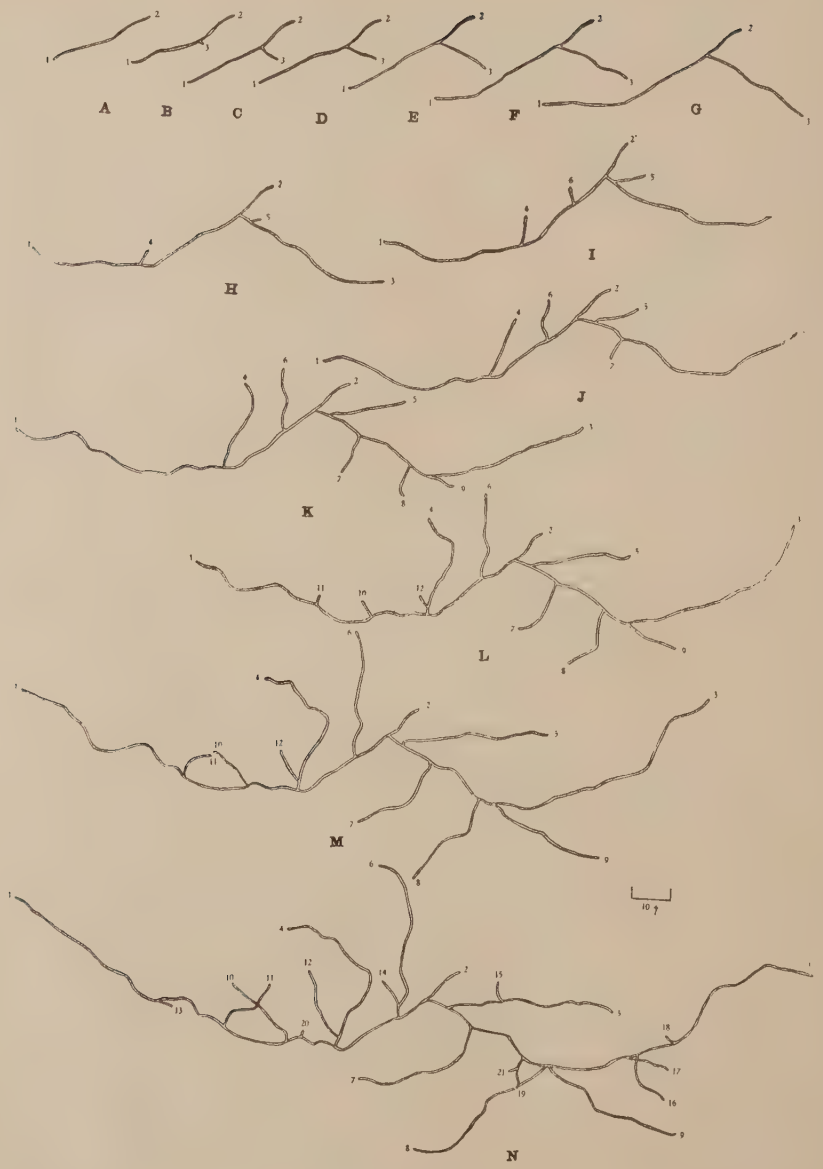
The colonial texture is waxy, and an insoluble intracellular pigment (cartridge buff, Ridgway Plate XXX 19 " F) is produced.

In Table 3 the developmental morphological characters observed in these studies are summarized.

TABLE 3.—*Summary of growth characteristics of eighteen strains of Nocardia.*

	Organism and plate number	Germination*	Primary Branching*	Secondary Branching†	Fragmentation				Number of Studies
					Age*	Types‡			
						1	2	3	
						Percentages			
GROUP I	W-F 1	8	16	0	13	70	30	0	7
	P. agnosus 1	6	12	0	12	60	30	10	16
	KLJ 2	9	15	0	13	50	50	0	14
	B-B 2	10	36	0	14	50	50	0	6
	N. erythropolis 3	11	14	0	14	60	40	0	11
	P. polychromogenes 3	11	13	0	14	40	30	30	9
GROUP II	13–20 4	11	12	5	19	6	28	66	16
	43–8 5	10	12	7	18	5	40	50	4
	P. ruber 6	10	14	4	20	5	30	65	27
	N. polychromogenes 7–8	14	30	20	5da.+	Unknown			6
	P. ast. var. *crat.* 9	10	28	4da.+	4da.+	Unknown			4
GROUP III	21–3 10	10	16	4		None			6
	13–10 11	9	11	6		None			7
	7–7 12	10	12	8		None			5
	18–2 13–14	11	15	6		None			14
	13–16 15	10	15	5		None			10
	13–3 16–17	10	13	9		None			11
	20–6 18	8	15	7		None			13

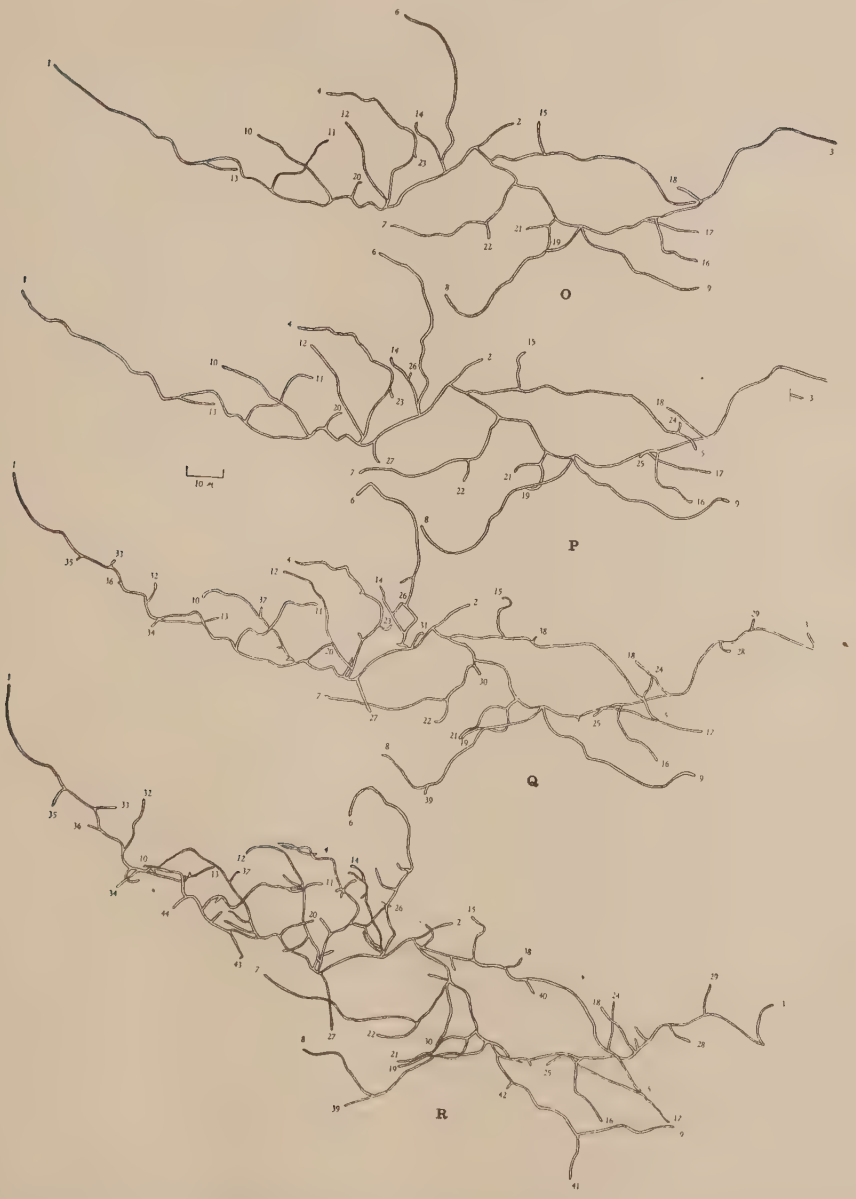
* Refers to the number of hours of incubation at 28° C.
† Refers to number of hours from primary branching.
‡ Refers to types of fragmentation described in the text.



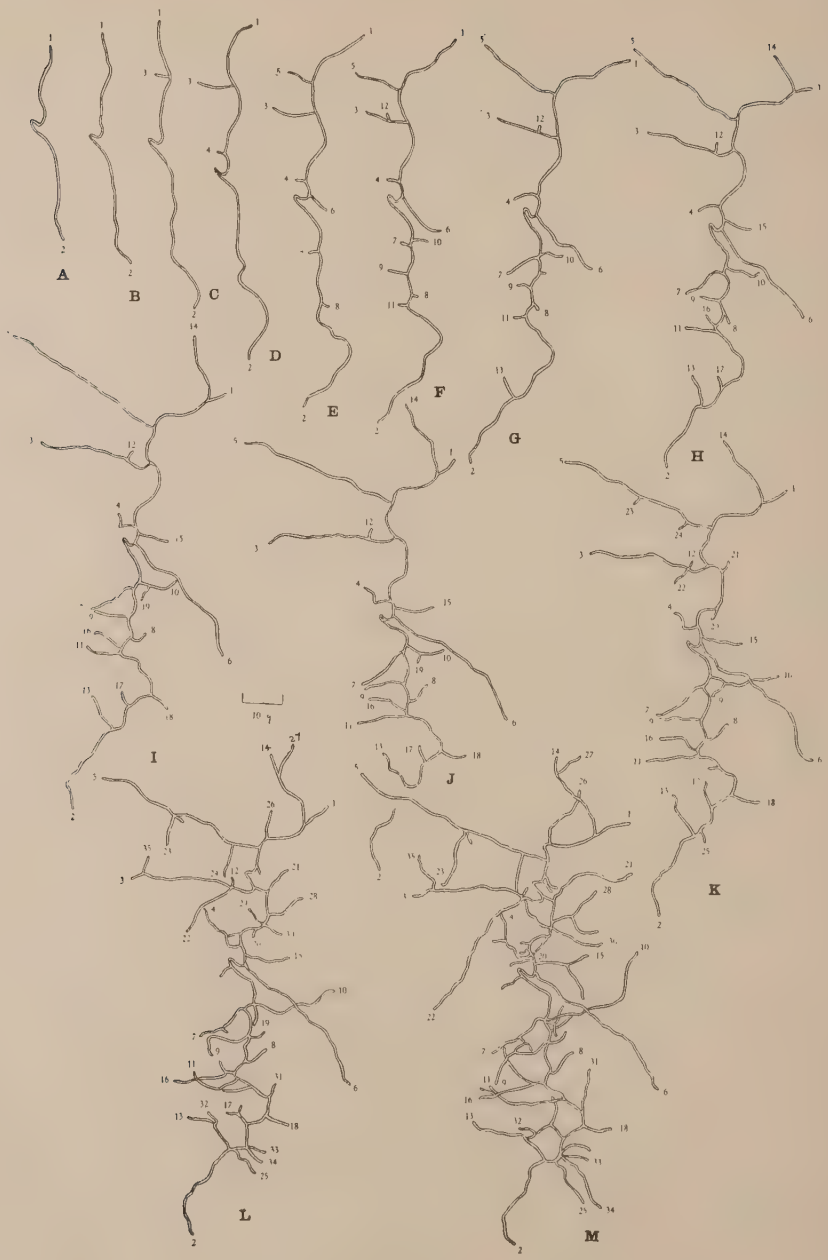
Plates 13 and 14

Strain 18–2. Glycerol nutrient agar; room temperature (circa 25° C). Study begins after 10½ hours of incubation. An 11-hour interval at 5° C separates figs O and P on Plate 14. Drawings are at hourly intervals.

The hyphal fragment in fig. A produces the first branch in fig. B after 11½ hours of incubation. Only end 1 grows, 2 remains the same length throughout. Branch 3 assumes the main development of the young colony. A secondary



branch 5, is soon produced in fig. H after 17 hours of incubation. Other branches are rapidly produced. The ends 1 and 3 grow out very rapidly, and branches are rather widely spaced. In figs. P, Q, and R the branches assume a more contorted appearance, and sharp bends as in 3 of fig. Q become more frequent. Branches cross freely, 10 and 11 of fig. N, for example, and sometimes grow down into the agar as represented by the end of 4 in fig. R.



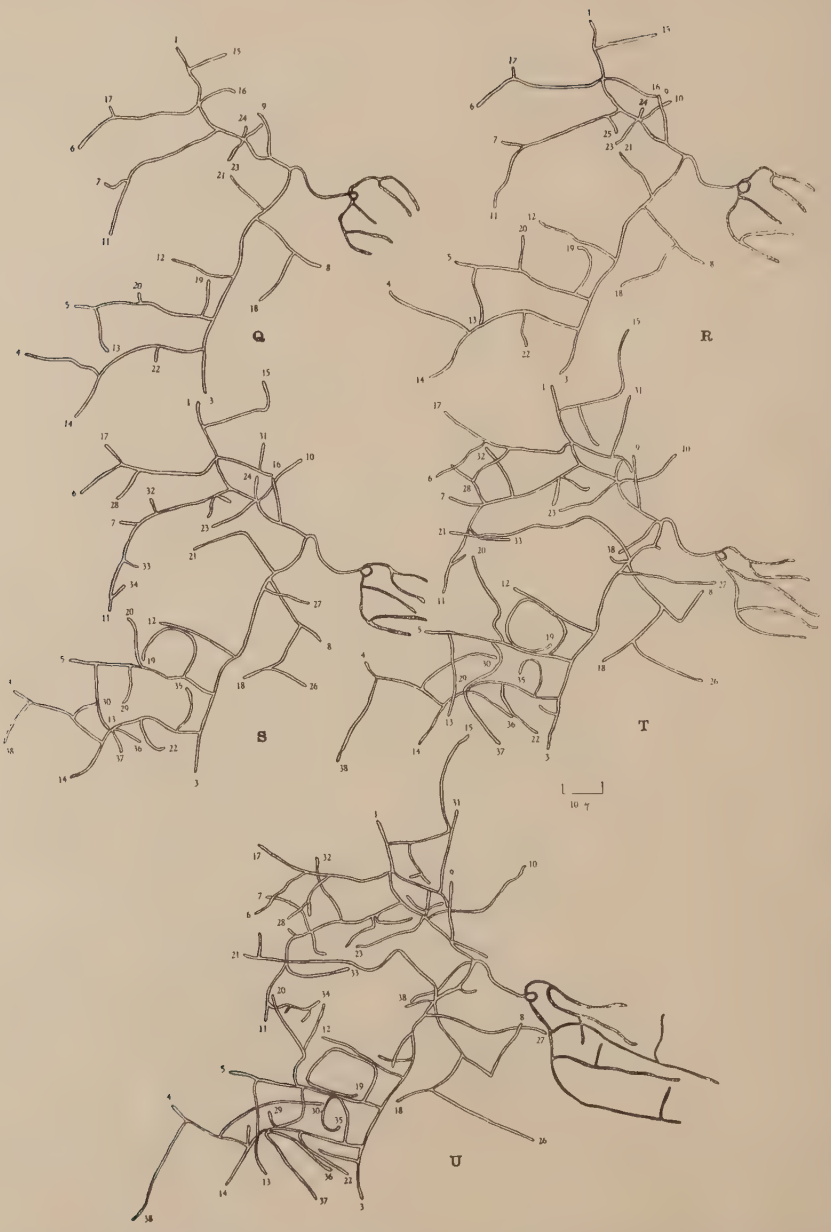
Strain 13–16. Glycerol nutrient agar; 28° C. Study begins after 14½ hours of incubation. A 10-hour interval at 5° C separates figs. K and L. Drawings are at hourly intervals.

The long hyphal fragment continues to elongate in figs. A and B, and produces a branch in fig. C. Other branches follow rapidly in subsequent figures. The parent hypha ceases to elongate in fig. J after 22 hours and branching becomes even more profuse. The first secondary branch 12, arises in fig. F, after 20 hours of incubation. The branches cross freely, and present a contorted appearance. Growth is rapid in this organism, and the branches are closely spaced.

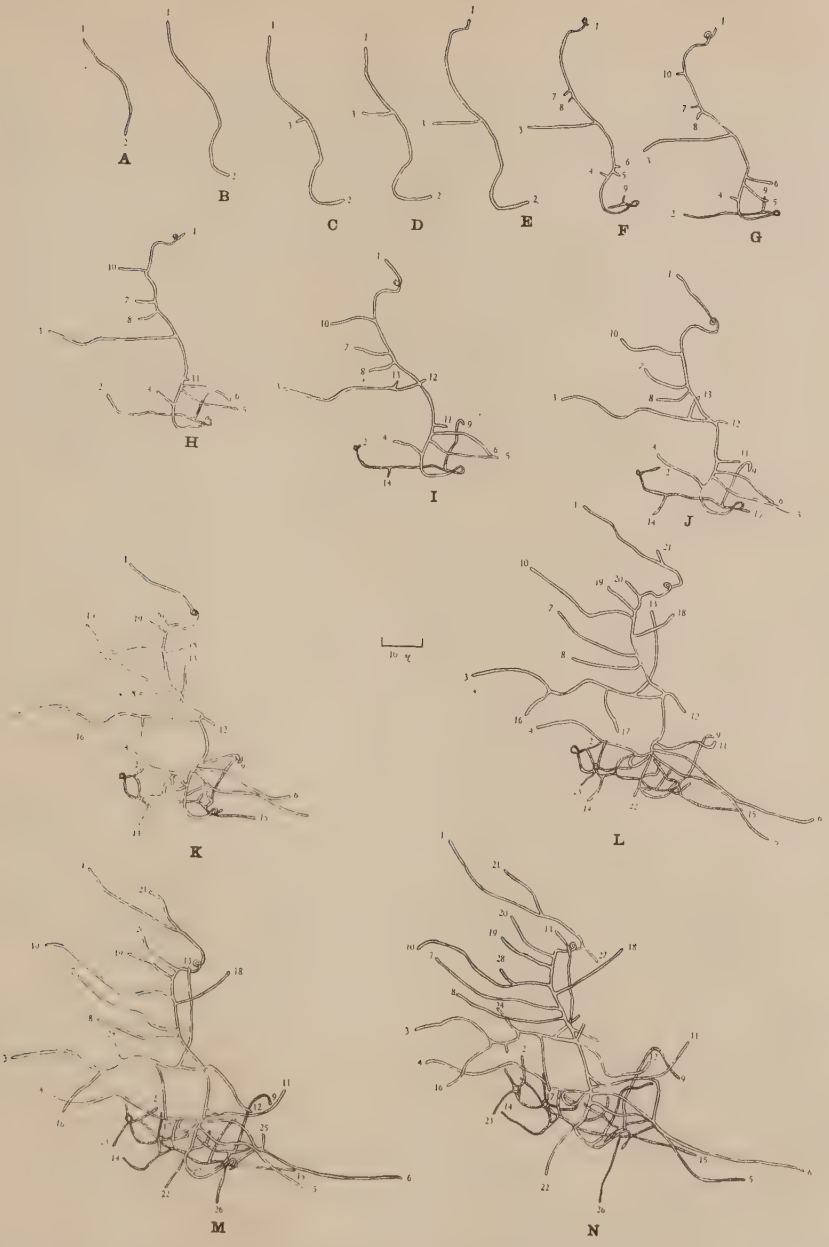
Plates 16 and 17

Strain 13–3. Glycerol nutrient agar; 28° C. Study begins after 11 hours of incubation. A 10-hour interval at 5° C. separates figs. N and O; and a 7-hour interval at 5° C separates figs. S and T, plate 17. Drawings are at hourly intervals. The unnumbered stippled hyphae are sub-surface.

The hyphal fragment grows from both ends in figs. A to M, at which time it ceases to elongate and branching becomes more profuse. The first primary



branch, 3, is produced at 12 hours in fig. B. It grows very rapidly and sets the pattern of the growth of the young colony. Branch 2 loops at figs. H and I and grows down into the agar at fig. J, where it continues to grow and branch. Branches tend to be widely spaced. The parent hypha does not become a complex of filaments, but some of the branches such as 4 and 5 in fig. U, form a dense growth. Branches, 19 and 35 in figs T and U, bend rather than grow across other filaments.



Strain 20–6. Glycerol nutrient agar; room temperature (circa 25° C). Study begins after 9 hours of incubation. Drawings are at hourly intervals. The stippled hyphae are sub-surface.

The hyphal fragment elongates from both ends, and primary branching begins with 3 in fig. C after 11 hours of incubation. The first secondary branch is 13 in fig. I, after 17 hours of incubation. The end of the parent hypha, 2, loops in a clockwise direction and grows down into the agar at fig. F. It subsequently branches, and complicates the growth pattern of the organism. Hyphal end 1, loops in a counter-clockwise direction at F, but does not grow down into the agar. Many other branches are produced which are somewhat contorted, and cross freely. Fig. N is the appearance of the young colony after 21 hours of incubation.

DISCUSSION

In the preliminary work sixty-one strains of actinomycetes were isolated from 23 different soil samples. The saprophytic, aerobic isolates are placed in the genus *Nocardia* on the basis of lack of spores in aerial hyphae, and the formation of a "mycelium." It was found in the course of this work that ten of these strains did sporulate, but sparsely and irregularly. The colonial texture of the isolates range from those that are soft and mucoid, through dry pasty forms, to those that are waxy and cartilaginous.

An extensive study of the number and distribution of *Nocardia* in soil has not been made. Jensen (1931) found that 2 of 73 colonies of actinomycetes from one soil sample were *Proactinomyces*. After incubating the soil with paraffin added, the number of *Proactinomyces* colonies increased to 30 of 303 colonies of actinomycetes. The present work indicates that although *Nocardia* are not found as frequently in soil as *Streptomyces*, they are present in appreciable numbers.

The most important morphological characteristics of actinomycetes were recognized by the first investigator to describe a member of the group. Cohn (1876, p. 186 *et. seq.*) describes the organism as follows: ". . . so kann man die Fäden . . . erkennt dann nicht bloss, dass dieselben sämmtlich von gleicher, so zu sagen haarfeiner Dicke, in unbestimmter Folge bald grad bald lockig gedreht verlaufen, sondern dass sie auch, wenn auch nur spärliche Verzweigungen zeigen." He also observed another important characteristic: "Die Fäden zerfallen in mehr oder weniger kleine Stücke, die mitunter kurz, oft aber 50 Mikrom. und darüber lang sind. . . ."

The most extensive studies of the morphology of actinomycetes are those of Drechsler (1919), on organisms which would now be placed in the genus *Streptomyces*. So far as the author is aware no studies have been made of the development of the mycelium of actinomycetes. The present studies of *Nocardia* indicate that these organisms go through a definite cycle of growth and development, which varies from strain to strain.

The organisms included in the genus *Nocardia* as it is now defined differ widely in their morphology. The forms which have a very rudimentary mycelial development, discussed here as Group I, correspond to the unstable mycelial group (*a* group) of Umbreit (1939). The very limited mycelium is the result of early fragmentation and sparse branching. The time of branching and fragmentation in a given strain was found to be relatively constant under controlled conditions. Because of the scant mycelial development, and the similarity of fragmentation to cell division in *Corynebacterium*, a possible relationship is indicated. It might be well argued that these organisms resemble bacteria more than actinomycetes. The separation of *Mycobacterium* and *Corynebacterium* from the ray fungi has always been a vague one. Umbreit (1939) states that the formation of "mycelium," an undefined term, is too indeterminate for an adequate separation. It is here suggested that the term "mycelium" be arbitrarily defined as a branched hypha at least 10 microns long. Using this definition, all the organisms in the present studies would possess a mycelium. However, this could probably be demonstrated under some conditions in *Corynebacterium*

and *Mycobacterium*. It is felt, nevertheless, that in *Nocardia* such hyphae are frequently present in young cultures so that quantitatively such a conception might serve as a taxonomically useful criterion.

With a delay in fragmentation, there is a great deal more mycelium produced in Group II. These organisms also conform to the *a* group of Umbreit (1939). However, the degree of mycelial development is much greater than in Group I. The differences in manner of fragmentation which are correlated with the amount of mycelium are real ones and seem to be related to the fact that organisms in Group II branch much earlier and always before they fragment, whereas in Group I, fragmentation often precedes branching.

Strains like *P. polychromogenes* (Vallée) stand in an intermediate position between Groups I and II on the basis of their mycelial growth and types of fragmentation.

The strains in Group III, (*β Proactinomyces*, Umbreit, 1939) are morphologically distinct because of their extensive mycelium, lack of fragmentation, and contorted branches. There are intermediates such as *N. polychromogenes* and *P. asteroides* var. *crateriformis*, between Groups II and III.

The reorientation of newly formed hyphal tips, following division, is a frequent occurrence. They either grow out parallel to each other, or curve and grow past each other. It is difficult to decide whether the reorientation is the result of mechanical forces set up by the bending prior to division, or whether there is an actual repulsion of hyphal ends. From a study of the behavior of such hyphae, it was found that they cross rarely, although their orientation would seem to make crossing likely. Instead they usually bend and grow from each other. It would seem, that if the direction of growth were determined by mechanical processes or by chance, the number of crossings would be greater. Further evidence for the presence of an actual repulsion is found in the branching habit of organisms of Group II. Branches cross very infrequently, although they often meet, bend, and grow parallel. (Pl. 7, Fig. I, 4 and 5.) Although the mere physical presence of another hypha may be enough to prevent crossing it is believed to be more probable that the hyphae actually repel each other. Generally, the branches of strains in Group III cross each other freely.

The morphological characteristics that we may regard as "primitive" or bacterial are: 1) early fragmentation, 2) sparse branching, and 3) fragmentation of type 1. As has been shown, these are very typical for Group I, uncommon for Group II, and absent in Group III.

The characteristics which are "advanced" or fungoid are: 1) absence of fragmentation, 2) profuse and contorted branching, 3) looping and crossing of hyphae, and 4) production of soluble pigments. These are absent in Group I, profuse branching occurs in Group II, and all are characteristic of Group III. Thus Group II shows properties that are intermediate between Groups I and III.

Studies of the developmental morphology of eighteen strains of *Nocardia*, then show forms which range from those that resemble certain bacteria in morphology to those which are like *Streptomyces* without aerial hyphae and spores. In the strains that are most like bacteria, Group I of this paper, the mycelium is sparsely developed and

fragmentation is characteristically type 1. From Group I through intermediate forms the mycelial development becomes more extensive. Group II is characterized by an extensive mycelial development, and fragmentation is predominately type 3. From Group II through intermediate forms mycelial formation becomes more extensive, and in Group III fragmentation does not occur. This group represents the most highly developed strains of *Nocardia*, and strains such as *P. asteroides* var. *decolor* which produces aerial hyphae, are very close to *Streptomyces*. Group III may to some extent represent degenerate or accidental species of *Streptomyces* as suggested by Shatz and Waksman (1945).

SUMMARY

1. Sixty-one strains of *Nocardia* (*Proactinomyces*) were isolated from twenty-three soil samples collected in the vicinity of Ann Arbor, Michigan; other cultures were obtained from type culture collections.

2. Morphological studies were made of the mycelial development by making serial drawings of one cell as it grew to a young colony. Eighteen strains were studied in this manner. Using mycelium development as a criterion the strains were separated into three morphological groups. Although individual strains may present similar pictures of development, there are often present constant differences which makes identification possible. For the most part individual strains in a group are difficult to distinguish on this basis; however, strains belonging to different groups are easily distinguished.

3. The fragmentation pattern in *Nocardia* can be separated into three types. In type 1, an acute angle is formed in a hypha preceding division, which occurs at the apex of the bend. Following division the new hyphal tips grow out parallel to each other. In type 2, division occurs in a straight or slightly curved portion of a hypha. Following division the newly formed ends bend slightly, and grow past each other. In type 3, division occurs in the parent hypha close to or at the juncture of a branch. A new hypha may grow from the place of division at the base of the branch. The newly formed hyphal tip bends and continues to grow. Type 1 fragmentation is characteristic of Group I, and type 3 of Group II. Type 2 occurs in both groups, and Group III lacks fragmentation.

4. The characteristics of the three groups of *Nocardia* are:

Group I: Scant mycelial development, sparse branching, and type 1 fragmentation. Colonial texture soft, pasty and sometimes mucoid, pigment intracellular and insoluble.

Group II: Extensive mycelial development, straight branches which do not overlap, and type 3 fragmentation. Colonial texture soft and pasty, pigment intracellular and insoluble.

Group III: Extensive mycelial development, no fragmentation of hyphae, contorted and profusely produced branches which overlap. Colonial texture waxy or cartilaginous. Generally both intracellular and soluble pigments are produced.

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The Rasāñjana of the Hindus

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The medicinal properties and uses of Rasāñjana were known in India long before 600 B. C. Suśruta, supposedly the earliest author of the Āyurveda, referred to it on many occasions for its efficacious medicinal properties and Caraka (1st Century B. C.) also recorded its manifold uses. Later authors, Vāgbhata (*c.* 3rd or 4th Century A. D.), Cakra Datta, Sarngadhara (*c.* 12th Century A. D.) and Bhāva Miśra (*c.* 14th Century A. D.), followed Suśruta and Caraka and mentioned some of its special uses.

Rasāñjana is the extract of the Hindu drug Dāruharidrā. In the northern part of India it is also called Rasaut. Royle[1] was probably the first to find out that Dāruharidrā is a species of Barberry (*Berberis*).

"While travelling in the Himalayas," Royle writes, "I continued my enquiries on the subject[2] and on wishing to be shown the plant which produced the wood called Dār-huld,[3] as well as that from which rusot[4] was produced, species of Barberry was immediately pointed out; and I was told that both the wood and the extract were procured indifferently from *Berberis asiatica, B. aristata, B. Lycium*,[5] as well as from *B. pinnata*, the *Mahonia nepalensis* of De Candolle."

Schoff[6] accepted this view in an annotation in his book,[7] "The Periplus of the Erythrean Sea," and interpreted Lycium as the extract Rasāñjana.

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[1]J. F. Royle. On the Lycium of Dioscorides. Trans. Linn. Soc. London **18**: 83. 1837.

[2]Of Rasaut and Dāruharidrā.

[3]Common name of Dāruharidrā.

[4]Rasaut.

[5]Royle found a new species of *Berberis* in Northern India. He called it *B. Lycium* in recognition of the Lycium of Greek medicine, which he recognized as Rasāñjana.

[6]W. H. Schoff, The Periplus of the Erythrean Sea, 1912.

[7]The book is an account of travel and trade in the Indian ocean by a merchant of the first century.

"The ships lie at anchor at Barbaricum,[8] but all their cargoes are carried up to the metropolis by the river to the king. . . . There are exported from these places,[9] spikenard, costus, bdellium, *lycium*, cotton cloth of all kinds, indigo, etc. . . . "

The preparation of the extract of *Berberis*, i.e., Rasāñjana, was described by Pliny.[10] The description reads like that of the preparation of Rasāñjana in Hindu medicine.

"The stems and roots which are intensely bitter are pounded and then boiled for three days in a copper vessel; the woody parts then removed, and the decoction boiled again to the thickness of honey."[11]

In the second century a duty was levied against this medicine, Lycium, at the Roman custom house of Alexandria. The extract was said to be preserved in little pots now found in collections of Greek antiquities. Many empty Lycium pots were found in the ruins of Herculaneum and Pompeii.[6]

It seems that Europeans learned about *Berberis* from the Arabs, for the knowledge of Greek medicine was transferred to the Arabs by means of translations made at Baghdad, and the Persians in turn translated this account from Arabic into their own language. The name *Berberis* was derived from the Arabic word Amburbaree, for turmeric colored wood. The Persian synonyms for it are Zerisk, Zaruj, Zuranj and Zurak, all having references to the golden color of *Berberis*, the synonyms apparently having originated from Zur, gold. One of the Sanskrit synonyms for Dāruharidrā is Sauvarni, derived from Suvarna, meaning gold. The Arabs knew about the extract also from the Hindus, for the author of Mukhzun-ool-Udwieh alluded to a writer who mentioned that Rasaut is the inspissated extract made from the decoction of the fresh wood of Dār-huld or turmeric colored wood. Dāruharidrā, probably the first name given to *Berberis*, also means yellow wood. The derivative meaning is 'woody turmeric,' signifying obviously to the bright golden yellow color of the stem of the plant.

The species of *Berberis* are shrubs, some of them attaining a height of six to seven feet, and sometimes even more. The girth of these tree like shrubs is about six inches in circumference. The Tibetans use poles of *Berberis* to stir boiling butter, in order that the latter may get a fresh golden color. The characteristic yellow color of the stem attracted the notice of the Āyurvedic druggists, who called it Haridrā, turmeric. It has also been described by other names of turmeric, Rajani and Niśā, with the prefix Dāru, wood. It is also called Pitadru and Pitadāru,

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[8]Probably a Hellenised name for Bandar, port.

[9]Barbaricum and Ozene, which is the modern Ujjayini, a trade center.

[10]Natural History, XXIV, 77.

[11]Rasāñjana is prepared by boiling dried root bark (1 part) of Dāruharidrā with water (8 parts) and concentrating the extract to one-fourth its weight. The concentrated extract is then filtered and the filtrate is boiled with an equal quantity of milk and concentrated to thick opium consistency.

"Dārvikvātha samam ksiram padam paktvā yadā ghanam. Tadā rasāñjanākhyam tat."—Bhāvaprakāśa, Purvakhanda, Part I.

both meaning yellow wood. In other languages the Sanskrit meaning of its different names has been retained.

It is not definitely known which species of Berberis yield Dāruharidrā of Hindu medicine. As the science of the Āyurveda developed in north-western India and in the United Provinces, it is quite likely that some of the species native in these areas represent Dāruharidrā. For it is known that Suśruta studied in Benaras under Rājā Divodās Dhanvantarī.[12] Carak-Saṃhitā is the revised and enlarged edition of the Agniveś-Saṃhitā.[13] Angiveśa (6th Century B. C.) who wrote a treatise on medicine was a pupil of Punarvasu Ātreya[14] of the University of Taxilla. Both Suśruta and Agniveśa must obviously have considered the species of *Berberis* growing in their immediate neighborhood. According to Royle,[1] wood and extract were obtained from *B. asiatica*, *B. aristata*, *B. Lycium*, and *Mahonia nepalensis*. This is highly probable for the present writer found that the same or allied alkaloids are present in the Indian species of *Berberis* and *Mahonia*, although in varying quantity.[15] Besides *B. asiatica* is the most widely distributed species in India.[16] Consequently Dāruharidrā with all its synonyms refers mostly to this species.

Watt[17] says that the yellow dye obtained from the root and stem of *Berberis* is perhaps one of the best tanning dyes in India. Probably it was also used for dyeing cloth, for the aqueous extract imparts a fast yellow color to wool. It was believed to be used by the Buddhists for their conspicuous yellow robes.

The chief uses in Hindu medicine of *Berberis* and its extract were recognized by the medical men of the last decade of the last century. Suśruta used root or bark of *Berberis* as the main ingredient of the recipes for cold, all kinds of skin diseases, eczema, ringworm, erysepelas, boils inside the stomach, for cleansing wounds, leprosy, fistula-in-ano, urine and stomach troubles, dysentery, bile complaints, worms, jaundice

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[12]Atha khalu bhagavantammaravaramrsigana parivrtamāśramastham Kāśirājam Divodāsam Dhanvantarīmaupadhenava Vaitaranaurabhra Pauskalāvata Karavīrya (ra) Gopuraraksita Suśruta prabhrtaya ucuh.—Susrut-Saṃhitā, Sutrasthānam, I, 3.

[13]Ityagniveśakrtetantre caraka pratisamskrte sutrasthāne . . . . . - Carak-Saṃhitā, Sutrasthānam, I.

[14]Atha maitrīparah punyamāyurvedam Punarvasuh śisyebhyo dattavān sadbhyah sarvabhutānukampaya.—Carak-Saṃhitā, Sutrasthānam, I, 30.

Agniveśaśca Bhedaśca Jatukarnah Parāśarah Hāritah Kśārapaniśca jagrhustanmunarvacah, *Ibid.*, I, 31.

[15]R. Chatterjee. The Alkaloid of *Berberis umbellata* Wall. Jour. Indian Chem. Soc. **17**: 289, 1940; **19**: 233 & 385: 1942; Umbellatine from *Berberis insignis* Hook. f. Jour. Amer. Pharmac. Assoc. **30**: 247, 1941; A Chemical Study of *Mahonia nepalensis* D. C. *Ibid.* **33**: 210, 1944.

[16]*B. asiatica* is distributed from East to West Himalaya, from Sikkim, Bhutan, Nepal to Garhwal and Bihar. The next largest area is occupied by *umbellata*. Of all species of *Berberis* now found in India, best known are *B. asiatica* and *B. aristata* of the Western Himalaya, and in the Eastern Himalaya *B. umbellata*. *B. asiatica* is found at an elevation of 3–11,000 ft., *B. aristata* between 6–10,000 ft. Both were discovered by Roxburgh in 1821, prior to the discovery of other *Berberis* species.

[17]G. Watt. A Dictionary of the Economic Products of India, I, 1889.

and eye diseases.[18] He also used the extract of *Berberis* as an antidote to poisoning, eye and ear troubles.[19] In addition to these uses Caraka mentioned it as a stimulant and as a remedy for oral ulcers and piles.[20]

The Bower Manuscript[21] excavated in Kuchar, Eastern Turkesthan, contains Navanītaka which brings together the best known formulae of the maharsis or medical authorities of the time. The date of this digest is about the second Century A. D. Navanītaka refers to the prevalent uses of *Berberis*, as the latter is considered to be a standard drug for skin and eye diseases, bleeding sores in the mouth, and abscess in the throat.[22] Cakra Datta mentions a special use of the decoction of the Rasanjana in menorrhagia and copious discharges from the womb.[23]

Vāgbhata mentioned special uses of *Rasanjana* in obesity, rheumatic pains and brain affections.[24] Sarngdhara, representing a much later period, used *Berberis* externally mainly in fever and in syphilis.[25] He probably was the first to use it in fever. As will be related later, even recent physicians got good results by using it in obstinate cases of fever. Actually from the time of Suśruta down to the 12th Century A. D., the extract of *Berberis*, either alone or in mixture, was used by different medical authorities and, notwithstanding the fact that Suśruta men-

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[18]Kaphapittārtināśanah kusthakrmiharaścaiva dustavranavišodhanah.—Suśrut-Samhitā, Sutrasthānam, XXXVIII, 65.

Stanyavišodhanau āmatisarasāmanau viśesaddosapācanau, *ibid.*, 28.

Gandamālāsu mandalesvatha mehisu ropanarthahitam . . .

Bhagandaravināśanam, *ibid.*, Cikitsāsthānam, VIII, 46.

Vimlāpānadrte vā'pi ślesmagranthi kramohitah, *ibid.*, XIX, 13.

Madhunā tarksyajam, *ibid.*, Uttaratantram, XII, 18.

[19]Tatpraśastham cirotthe'pi sāśrāve putikarnake, *ibid.*, Uttaratantram, XXVII, 50.

Raktapittānirvahanah visopaśamodaham nihantyābhyantaram bhrsam, *ibid.*, Sutrasthānam, XXXVIII, 42.

Nadivranāpahāh, *ibid.*, Cikitsāsthānam, VIII, 42.

[20]Arśoghnāni, Carak-Samhitā, Sutrasthānam, XIV, 12.

Kandughnāni, *ibid.*, XIV, 14.

Mukhaśodhanāh, *ibid.*, Cikitsāsthānam, VIII, 138.

[21]A. F. Rudolf Hoernle. The Bower Manuscript. 1893.

[22](Kan)tharogapraśamanah rasāñjanam dāruharidrikā . . . *Ibid.*, II, 41.

[23]Dārvyādikvāthah.

Dārvirasānja vrsāvda kirata vilva bhallātakai ravikrto madhunā kasayah Pitojayatyativalam pradaram saśulam pitasituna vilohita nilaśuklam, Cakra Datta.

[24]Amatisaranāśanau Medah kaphādhya pavana stanyadosa nirvahanau, Astāngahrdaya, 1941, 36.

Tarksyaśailam trutau prthvikā sodhayamtyuttamāmgam, *ibid.*, 4.

[25]Tailamamgārakam nāma sarvajvaravimoksanam, Sārngdhar-Samhitā, 1924, 98.

Rasāñjanam śirīsena pathyaya ca samanvitam, *ibid.*, XI, 104.

Saksaudram lepanam yojyamupadamsagadāpaham, *ibid.*, 105.

tioned its manifold uses, experiences of later authors enabled them to add to Suśruta's list new and important uses of *Berberis*.

Syphilis was not known in ancient India, as it was apparently introduced by Portuguese sailors and merchants, and became known as Phiranga Roga.[26] No wonder, therefore, that Rasāñjana earned a good name and found its way to Europe.[27]

Modern experiments on the therapeutic uses[17] of *Berberis* and its extract were first made by Royle,[28] who used the extract with success, both simply and in combination with opium and alum, in chronic inflammation of the eye.[29] Sheriff described Rasāñjana as one of the few good drugs of Hindu medicine. He found that an aqueous extract of the powdered root of *B. aristata* was a very inferior preparation and very indifferent in its action. He, therefore, gave the different methods of preparation of decoction, tincture and watery extract of its root. He used the tincture as a bitter tonic, stomachic,[30] cholagogue, alterative and antiperiodic.[31] Anderson found that Rasāñjana, mixed with opium and lime juice, is a most useful external application in painful eye affections. Parker spoke highly of its use in indolent ulcers[32] and Banerjee found that the tincture is useful in cases of enlargement of the liver or of the spleen. O'Shaughnessy described Rasāñjana as a febrifuge.[33] Due to its action on liver, the drug increased appetite, promoted digestion and acted as a very gentle but sure aperient. Lal used it both locally and internally in piles. Penny prescribed it in dosages of 5 to 15 grains for bleeding piles and as a wash in solution.[34] Its ointment made with camphor and butter was applied to pimples and boils. Barren followed Vāgbhata[34] by stating that a decoction of *Berberis* was useful in scarlet fever and brain affections. Its use as a healing ointment in indolent ulcer prompted Jolly[35] to try dried Rasāñjana in the treatment of oriental sore. The yellow active principle of the Indian species of *Berberis* is either berberine or umbellatine; both alkaloids are good cures of oriental sore, and berberine is still a specific for it.

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[26]A disease of the Portuguese or foreigners.

[27]It (the decoction of *Berberis*) is mixed with various bitter extracts and with a murca of olive oil and ox-gall. The froth of this decoction is used as an astringent in compositions for eyes, and the other part as a face cosmetic and for the cure of corroding sores, fluxes, and suppurations, for diseases of the thorat and gums, for coughs, and locally for dressing open wounds. Pliny, *loc. cit.*

[28]J. F. Royle, "An Essay on the Antiquity of Hindoo Medicine," 1837.

[29]Cf. Suśruta.

[30]Cf. Caraka.

[31]Cf. Sārṇgadhara.

[32]Cf. Suśruta.

[33]Cf. Sārṇgadhara.

[34]Cf. Caraka.

[35]G. G. Jolly, The Treatment of Oriental Sore, *Indian Med. Gaz.* **46**, 466, 1911.

The Occurrence of a Green Sulphur Bacterium in Sodon Lake

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In August of 1947 a green sulphur bacterium appeared in tremendous numbers in the phytoplankton of Sodon Lake, Bloomfield Hills, Michigan. Sodon Lake, at its greater depths, is moderately rich in hydrogen sulphide and affords a habitat suitable for the growth of green bacteria, i.e., an anaerobic condition and a favorable response by the bacteria present in this oxygen-less zone to low light intensity. An initiatory reconnaissance on the organism was started by the staff of the Cranbrook Institute of Science and continued by the author from January, 1949,[1] throughout the following summer. In connection with a bacteriological survey currently in progress on the ecology, distribution and nutrition of other aquatic bacteria of Sodon Lake, we have studied the vertical and horizontal distribution, the culture, chemical and biological environment and morphology of the green bacterium. In as much as green bacteria are of considerable importance in investigations on the fundamental nature of photosynthesis (see especially van Niel, 1949) and since the clarification of their taxonomical status depends upon culture studies, a progress report is indicated here.

The organism has been tentatively identified as *Chlorobium limicola* Nadson. Another organism, *Pelogloea bacillifera* Lauterborn, with similar characteristics has been reported from Scaffold Lake, Wisconsin by Dutton and Juday (1944).

The organism was isolated by the single cell isolation technique and also by the use of enrichment cultures. In the latter case, repeated dilutions of the crude culture gave one that appeared microscopically to consist of one type of cell. The culture medium used was suggested by van Niel (1931) and modified by the substitution of aged, centrifuged lake water to replace the distilled water of van Niel's medium. This substitution was made for the purpose of incorporating into the medium any trace nutrients that may still have been present in the lake water.

The bacterium appeared as a slightly elongated non-motile rod about one μ by 0.5 μ (Fig. 1); however neither shape nor size are constant morphological factors. Cell conglomerates are present and the cells are always surrounded by capsular material which can be dissolved off by suitable bacteriological techniques. Electron microscope pictures[2] show 2–3 areas of heavy condensation, approximately 0.25 μ in diameter as determined by reference to particles of polystyrene latex, the interpretation of which must await further study.

The seasonal appearance of the organism has been erratic. It was first noted in the phytoplankton during February, 1949, and remained

[1]With the assistance of Stanley A. Cain, F. Segadas-Vianna, and Floyd Bunt, Cranbrook Institute of Science.

[2]We are indebted to R. Backus, Department of Physics, University of Michigan, for his assistance in this work.

present, although in diminishing numbers, until April. The organism did not reappear in the phytoplankton until July, at which time its vertical and horizontal distribution was determined. The zones of concentration were determined by sampling from stations selected according to the contours of the lake at 56 ft., 50 ft., 40 ft., and 20 ft. depths. Microscopical examination of the phytoplankton at 5 ft. vertical intervals indicated that almost a pure culture of green bacteria existed at 25 ± 5 ft. This observation was further tested by determining the absorption spectra of the various samples in a Coleman Jr.

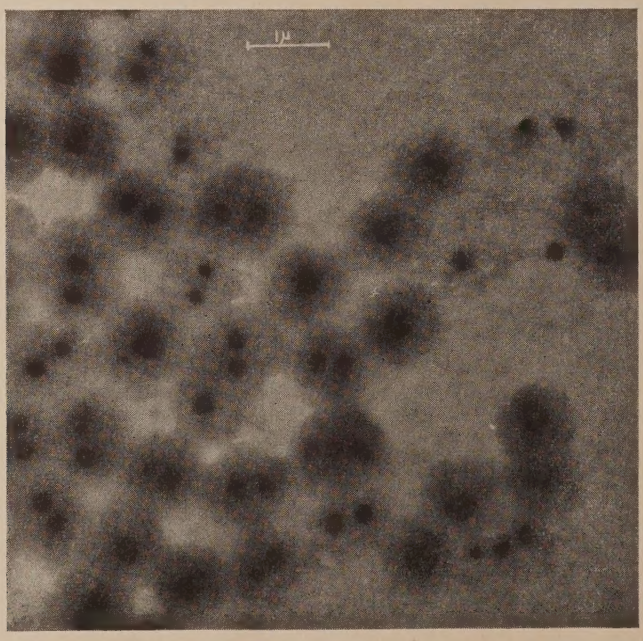

FIG. 1. Green bacterium, showing morphology and areas of condensation.

spectrophotometer. In every case the zone of concentration is sharply delimited at 25 ± 2 ft. The horizontal distribution was determined over a distance of 175 ft., again extending from the 56 ft. depth to the 20 ft. depth. Here, also the zone of concentration appeared to be at 25 ± 2 ft. and extending shoreward to meet the lake bottom.

It is usually stated that green bacteria develop in an environment rich in H_2S. By actual determination the amount of H_2S in the zone of concentration is very small and frequently a positive test for this substance can not be obtained. While H_2S originates in the bottom deposits and is obvious by odor and by chemical tests below the zone of Chlorobium concentration, it is apparently being removed by the bacteria in their zone of concentration.

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